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(AGRICULTURAL RESEARCH SECTION)

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NOTICES

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THE METABOLISM OF SULPHUR IN THE RUMEN OF THE SHEEP

By C. M. ANDERSON, Canterbury Agricultural College, Lincoln

(Received for publication, 25 August 1955)

Summary

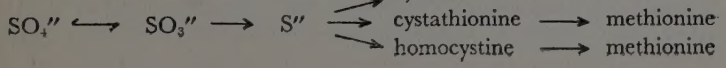
The fate of sulphate, sulphide, and protein-sulphur has been studied *in vitro* in washed suspensions of ruminal microflora, and *in vivo* in the rumen of the sheep.

The down-grading of sulphate, protein-sulphur, and "free-amino-acid sulphur" to sulphide by the ruminal microflora, the consequent utilization of this by the sheep, and the up-grading of sulphide to microbial protein, are discussed and shown to depend upon the conditions obtaining in the rumen.

INTRODUCTION

The importance of sulphur in the nutrition of the ruminant, or of any animal, needs no emphasis, but knowledge of how the ruminant utilizes the various forms of sulphur to be found in plants is limited.

Warth and Krishnan (1935) have shown that inorganic sulphate fed to sheep resulted in an increase in the organic sulphur content of the rumen and in increased organic sulphur in the urine, indicating that inorganic sulphate plays a part in the metabolism of the animal. The microbiological synthesis of methionine from inorganic sulphur has been described by Loosli *et al.* (1949), and Duncan *et al.* (1953) and Block and Stekol (1950) showed that labelled sulphur given orally to a cow, as sodium sulphate, was incorporated within three hours in the cystine and methionine of the milk proteins. Later, Block *et al.* (1951) reported that orally administered, labelled sodium sulphate was incorporated in the milk proteins of the goat, and also in the methionine and cystine of the ruminal protein of the ewe. They concluded that these two amino acids are synthesized by the microflora of the rumen utilizing the inorganic sulphur. They tentatively described the conversion of inorganic sulphur to cystine and methionine by the microflora as follows:—



The evidence for this conversion is slight, however. Lewis (1954) has shown that sulphate is reduced to sulphide in the rumen of the sheep, and also *in vitro* by washed suspensions of ruminal contents, using as hydrogen donors gaseous hydrogen, glucose, pyruvate, succinate, and other tricarboxylic acid cycle components. He has also shown that sulphite and thiosulphate are reduced to sulphide *in vitro*, and that sulphide can be released from l-cysteine by washed suspensions of ruminal microflora. Hale and Garrigus (1953) have described studies in which labelled sulphur from orally administered sulphate and elemental sulphur has appeared in the cysteine of the wool of the sheep. Starks *et al.* (1953) showed that lambs on a sulphur-deficient diet lost weight rapidly through inability to use dietary nitrogen and also that their rate of growth of wool was decreased.

The present work is a study of the utilization by the ruminal microflora of various sulphur-containing compounds, such as protein and inorganic sulphate and sulphide, and an endeavour to correlate these with the equivalent compounds normally found in plants.

METHODS

In Vivo Experiments

The experimental sheep, a ewe, was fitted with a permanent rumen fistula. It was fed *ad lib.* on lucerne hay containing 0.28% total S. When the *in vivo* experiments were in train, the sheep was fed at 7.30 a.m. and removed at 9.00 a.m. to a bare pen. An hour and a half later the initial sample of ruminal contents was withdrawn and the substance to be studied was introduced. At each appropriate withdrawal time, small random samples of the ruminal contents were taken, mixed together, and filtered through muslin. The filtrate was then used for the analysis.

In Vitro Experiments

In these, washed suspensions of ruminal microflora were prepared by the method of Sijpesteijn and Elsden (1952), except that no sulphide was added to the washing solution. The phosphate buffer was boiled and cooled rapidly just before use and, where possible, the substrate solution was also quickly boiled and cooled, or else made up in recently boiled distilled water. Ruminal contents taken from the sheep, three to four hours after feeding, were used to prepare the washed suspensions. These were made up in a calculated volume of buffer solution, so that later addition of the solution containing the substrate would bring the total volume to that of the original ruminal liquor.

The reactions were carried out in Warburg vessels with double side-bulbs, at 37°c. under an atmosphere of N_2. The solutions incubated contained 2 ml. of the washed suspension, 0.5 ml. of a 0.1 M glucose solution, and 0.5 or 1 ml. of substrate solution. Any sulphide produced by the reaction was absorbed in the centre well by alkali, which was carefully removed later to a glass-stoppered flask for estimation of sulphide. When sulphide was the substrate, the alkali was placed in an empty side-bulb after the incubation had proceeded for the desired period of time.

Sulphide Estimation

Sulphide was determined by the method described by Lewis (1954), using the reaction $H_2S + I_2 = 2HI + S$, in an acidified aqueous solution, and depending upon the colorimetric estimation of iodine by the purple colour it exhibits in a carbon tetrachloride solution. The following modifications were used:

(1) Percentage transmittance values were used, at minimum transmittance 515 mμ. The values for iodine concentrations were in microgrammes.

(2) The initial iodine in carbon tetrachloride solution was standardized in the same way, by shaking with a known excess of $Na_2S_2O_3$ and back-titrating with a standard iodine solution. This I_2/CCl_4 solution was used for building the standard transmittance curve, but the iodine concentrations of subsequent standard solutions, or dilutions of them, were obtained from the standard curve.

(3) Instead of separating funnels, 250-ml. glass-stoppered flasks were used for the estimation. Into the flask, containing the aqueous sulphide solution, was run from a burette 25 ml. of the standard I_2/CCl_4 solution. Without loss of time, the contents were acidified and the flask stoppered and shaken vigorously for 3 to 4 minutes. The aqueous layer was then removed and the percentage transmittance of the I_2/CCl_4 solution measured in a Coleman spectrophotometer, model 14. To an aqueous solution containing the same amount of alkali but no sulphide, was added 25 ml. of I_2/CCl_4 standard. This was similarly treated as above, and the resulting value for standard I_2 concentration used as the true one for the purpose of estimating sulphide.

The sulphide concentration of ruminal liquor was estimated by microdiffusion in Conway units, from 2 ml. of filtered ruminal liquor in the outer compartment into 1 ml. of 10% KOH in the inner well. After acidification of the ruminal liquor by 0.5 ml. 4N HCl, the vessels were allowed to stand at room temperature over-night, or for 24 hours. The alkali was then removed and the sulphide estimated as described.

Sulphate Estimation

Sulphate sulphur, protein sulphur and "free-amino-acid sulphur" were all determined as $BaSO_4$, the latter two first being converted to sulphate either by the $MgNO_3$ method (Ass. Offic. Agric. Chem. 1945), or by a mixture of nitric and perchloric acids. In the analysis of the ruminal contents for the above components, a volume (100 ml.) was withdrawn and filtered through muslin. Duplicate samples of 25 ml. were then used for the analysis. The 25-ml. sample was pipetted into 25 ml. of a solution of 4% sulphur-free tannin in 1% HCl and allowed to stand at least 3 hours. It was then filtered through Whatman 40 filter paper, the proteinaceous residue being washed several times with cold 2% HCl before being transferred completely to the filter paper, where it was again washed several times with the acid solution. The residue, together with the filter paper, was then oxidized and the sulphur content determined as $BaSO_4$ gravimetrically. The protein-free filtrate,

containing inorganic sulphate and "free-amino-acid sulphur", was then analysed for inorganic sulphate by the standard procedure of precipitating as $BaSO_4$ with 10% $BaCl_2$. This $BaSO_4$ precipitate, on filtering, was first washed with hot dilute HCl to remove any barium salts of the amino-acids, then washed with hot water and finally ignited in a weighed porcelain crucible.

The combined washings and filtrate from the inorganic sulphate precipitation, which now contained only the "free-amino-acid sulphur", were evaporated to dryness or near-dryness. A mixture of nitric and perchloric acids (Johnson *et al.* 1943) was then added to oxidize the sulphur of cystine and methionine to sulphate. This was then determined gravimetrically as $BaSO_4$. Recoveries of sodium sulphate added to ruminal liquor were 96–100%, whilst recoveries of added cystine (as "free-amino-acid sulphur"), were within the range 96–110%. Recovery figures in excess of 100% were probably due to partial hydrolysis of the proteinaceous residue during washing.

RESULTS

pH Optima

The optimum pH of washed suspensions for the production of sulphide from sulphate and protein, and also for the uptake of sulphide by the micro-organisms was determined. Sulphate reduction was not very sensitive to pH but had an optimum at 6.5. Both release of sulphide from protein (casein) and sulphide uptake were more sensitive to pH, each having an optimum at pH 6.0.

Sulphate Reduction in Vitro

Before studying the fate of sulphate *in vivo* it was deemed advisable

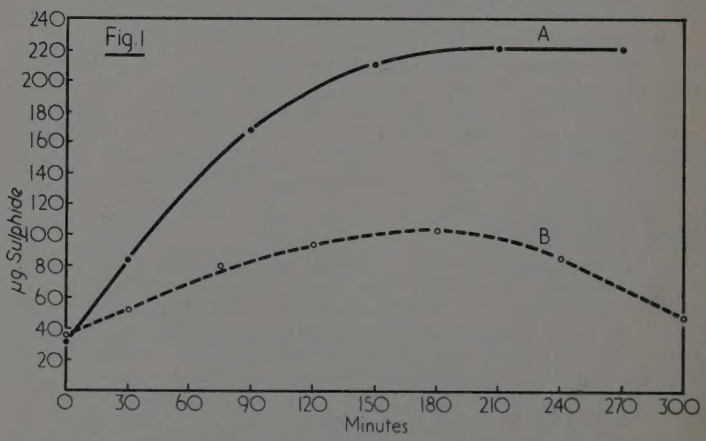


Fig. 1.—The fate of sulphate added to washed suspensions at pH 6.5.
A: 1 ml. of washed suspension, 0.5 ml. of a 0.1 M glucose solution, and 1 ml. of a sulphate solution equivalent to 500 μg. S/ml.
B: 1 ml. of a washed suspension, 0.5 ml. of a 0.1 M glucose solution, and 1 ml. of a sulphate solution equivalent to 200 μg. S/ml.

to carry out experiments with washed suspensions *in vitro* at pH 6.5. A selection of typical curves is shown in Fig. 1.

Curve A, resulting from an incubation with $Na_2SO_4.10\ H_2O$, equivalent to 500 μg. sulphur, shows a production of sulphide at a rate of 84 μg. S/hour, until at about 3½ hours from the beginning of the experiments the curve reaches a level at 220 μg. sulphur, where there is no apparent further production of sulphide.

Curve B, resulting from an incubation with $Na_2SO_4.10\ H_2O$, equivalent to 200 μg. sulphur, shows a production rate of sulphide of approximately 26 μg. S/hour until it reaches a peak at 104 μg. sulphur after three hours, after which the curve shows a disappearance of sulphide at the rate of 28 μg. S/hour.

It appears therefore that, in the first incubation containing the higher concentration of sulphate, the rate of sulphate reduction was greater initially than the rate of sulphide utilization until near the end of the incubation the latter rate increased, both rates finally becoming approximately equal. In the second incubation containing the smaller concentration of sulphate, the rate of sulphate reduction was at a maximum initially, and then either ceased or slowed down so that the utilization of sulphide became dominant.

Direct determinations on the amounts of sulphate remaining during the experiments were not carried out.

Reaction Rates

Studies, made with washed suspensions, to determine how fast sulphate, protein, and sulphide are attacked by the microflora, were carried out in Warburg vessels at various concentrations of the substrates, and at two different incubation periods of thirty and sixty minutes respectively. Since it was not possible to study the fates of the three substrates simultaneously, a stock washed suspension of microflora was stored under an atmosphere of carbon dioxide at 4° to 5°c. and used as required for the separate studies with sulphide, sulphate, or protein (casein). Table 1 shows the results of one such series of incubations; (a) is the initial concentration of substrate in μg. sulphur and (x) is the decrease or loss of substrate in μg. sulphur, after a lapse of time (t). The experimentally obtained sulphide values, shown in the table, have been corrected for the appropriate control blanks for sulphide which were 8, 12, and 15 μg. sulphur for the sulphide, sulphate, and protein studies respectively. The initial sulphur contents of the substrates were determined in each case on stock solutions of them.

It can be seen from the a/a-x columns in the table that none of the reactions fit the equation for a first-order reaction, i.e., $-dc/dt = kc$. However, on inserting the obtained sulphide values (x) in the equation for a zero-order reaction, i.e., $-dc/dt = k$ or $k = x/t$, where k is the reaction rate, it is found that the k's for each set of incubations, at the specified times, are in better agreement. This shows that the rate of uptake of, or release of, sulphide is independent of the initial concentration of the substrate and is dependent only on the bacterial population.

TABLE 1. REACTION RATES OF SULPHIDE UTILIZATION, AND OF SULPHIDE PRODUCTION FROM SULPHATE AND PROTEIN

	30 Minutes Incubation					60 Minutes Incubation			
	a μg.S	x μg.S	a-x μg.S	a/a-x	k = x/t μg.S/Sec.	x μg.S.	a-x μg.S	a/a-x	k = x/t μg.S/Sec.
Sulphide incubation	184	18	166	1.11	1.0×10^{-2}	44	140	1.31	1.22×10^{-2}
	92	20	72	1.28	1.11×10^{-2}	42	50	1.84	1.17×10^{-2}
	49	15	31	1.48	0.83×10^{-2}	40	(6)	7.7	1.11×10^{-2}
Sulphate incubation	250	30	220	1.14	1.67×10^{-2}	72	178	1.40	2.00×10^{-2}
	125	35	90	1.39	1.95×10^{-2}	75	50	2.50	2.08×10^{-2}
	63	27	36	1.75	1.50×10^{-2}	66	(0)		1.83×10^{-2}
Protein incubation	196	26	170	1.15	1.44×10^{-2}	32	164	1.19	0.89×10^{-2}
	98	28	70	1.40	1.56×10^{-2}	30	68	1.42	0.83×10^{-2}
	49	25	25	1.96	1.39×10^{-2}	28	21	2.07	0.78×10^{-2}

The table further shows that sulphide can be utilized quite rapidly by the ruminal microflora and explains the previous *in vitro* sulphate incubations. In the sulphide incubations the reaction rate has increased in the second period, indicating an increased utilization of sulphide. Similarly, the reaction rates for sulphate reduction indicate an increased reduction of sulphate. The reaction rates for the protein incubations show that at 60 min. the release of sulphide from protein has slowed down and nearly ceased. The reaction rates for the various substrates are not strictly comparable, because of the possibly harmful effect on the bacterial population of chilling and storing the stock washed suspension. But on the basis of a small series of simultaneous incubations with the substrates which indicated that the bacterial population in a suspension did not change greatly with the over-night chilling given, the rates may be considered roughly comparable; therefore Table 1 shows that, with the washed suspension used and at the concentrations used, sulphate is reduced to sulphide at a faster rate than that at which sulphide is utilized by the micro-organisms, and that protein sulphur is released at a rate intermediate between the former two.

It is recognized that the above data hold good only for the particular conditions of substrate concentrations and bacterial population studied, and that under other conditions of substrate concentrations, and especially bacterial populations, different results may be obtained.

Fractionation of Washed Suspensions Incubated with Sulphate

Incubations of large volumes of washed suspensions with sodium sulphate were carried out in order to determine, if possible, the fate of the sulphide produced from the sulphate.

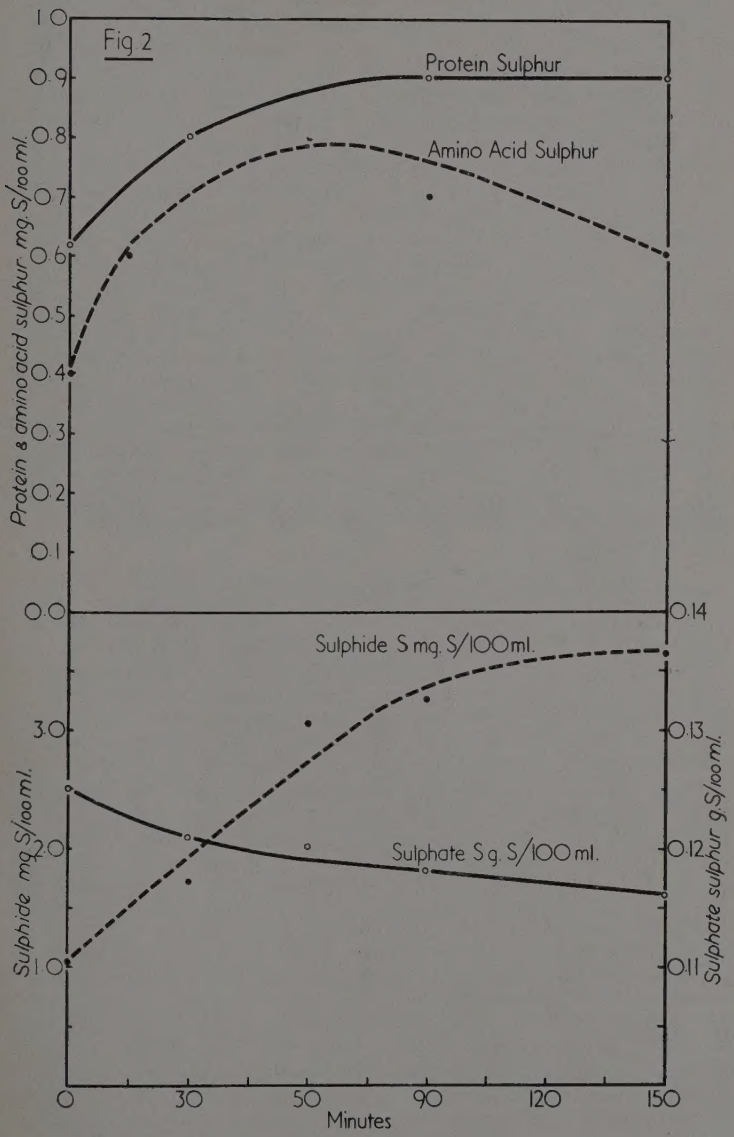


Fig. 2.—The fractionation of a washed suspension of ruminal microflora incubated with $Na_2SO_4.10\ H_2O$.

18.8 g. sodium sulphate dissolved in boiled distilled water were added to a suspension of ruminal microflora in phosphate buffer pH 6.5. A glucose solution and an ammonium chloride solution, adjusted to pH 6.5, were also added; the final concentrations of these being 0.05 M with respect to the total volume of 1500 ml., which was the same as that of the original ruminal liquor. The solution was incubated in a two-litre flask, under an atmosphere of carbon dioxide, at 37°C. It was gently swirled from time to time, and at intervals samples were taken for analysis. Fig. 2 shows the results of one such fractionation. All the data are in terms of grammes or milligrammes of sulphur per 100 ml. of suspension. The sulphide figures are scaled up to read in mg. S/100 ml. from the figures originally obtained as μg. S/ml. As can be seen from the curves, at the end of the incubation after 2½ hours there has been a loss of 9 mg. of sulphate sulphur and gains of 2.6 mg., 0.2 mg., and 0.16 mg. of sulphide, protein sulphur, and "free-amino-acid sulphur" respectively. These total 3.0 mg. of sulphur; there are still 6 mg. of sulphur unaccounted for, but, since only the sulphide in solution was determined, this loss in sulphur may be due to undetermined sulphide in the atmosphere of the flask which was lost every time the flask was opened. Approximate calculations based on the partial pressure of hydrogen sulphide in the atmosphere above aqueous solutions indicate that about 7 or 8 mg. of sulphur could be lost from the flask in this way. The curves indicate a reduction of sulphate to sulphide, with subsequent utilization of some of the sulphide produced by the microflora to produce new microbial protein.

Fate of Sulphide Added to the Rumen

Solutions of $Na_2S.9H_2O$ in distilled water, estimated on the basis of a four-litre rumen to be equivalent respectively to 37 μg. and 113 μg. S/ml. of ruminal liquor, were introduced into the rumen in two separate experiments in order to determine the rate of disappearance of sulphide. During the last experiment, an attempt was made to determine the concentration of sulphide in the blood, which was taken from the jugular vein. The determinations were carried out in Conway units by micro-diffusion from 2 ml. of acidified, fresh, oxalated whole blood in the outer compartment into 10% potassium hydroxide in the centre well. Fig. 3 shows typical curves for the disappearance of sulphide in the rumen. The initial concentration in the rumen, before addition of the 113 μg. sulphide solution was 18 μg. S/ml. and it can be seen from the figure that most or all of the added sulphide disappeared within two hours and over half of it in the first thirty minutes. At the same time there was an increase in blood sulphide of 6 μg. S/ml. which was maintained over a twenty-five minute period, the sulphide level then returning to zero within the hour. Under normal feeding sulphide could not be detected in the blood. The pH of the rumen did not vary much over the first two hours of the experiment (pH 6.36 – 6.44), and at this pH most of the sulphide should be in the un-ionized form of hydrogen sulphide. There appears to be a rapid absorption of hydrogen sulphide through the ruminal epithelium, the rates of disappearance over the first thirty minutes and over the second thirty minutes being about 174 μg. S/ml./hour, and 30 μg. S/ml./hour for 113 μg. S/ml. added to the rumen. Whilst the blood sulphide values are not considered to be particularly accurate, they do show a significant increase in the concentration of sulphide in the blood, and from this and the swift

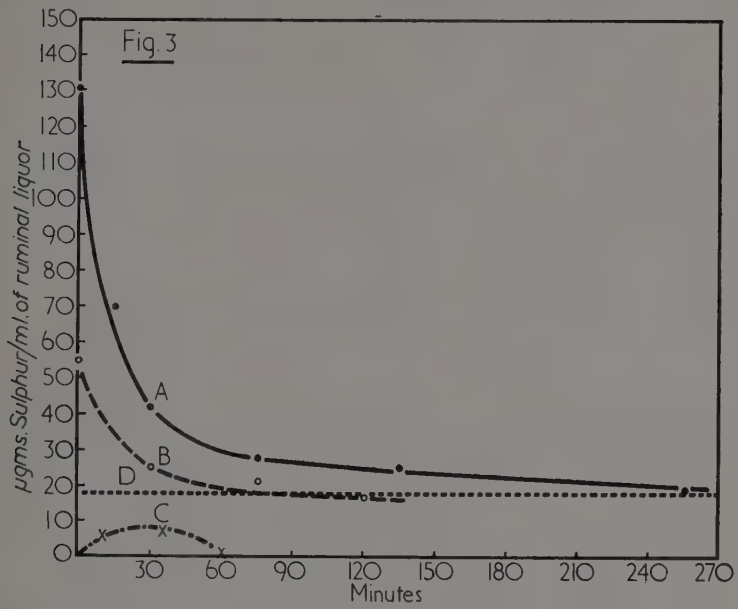


Fig. 3.—The fate of $Na_2S.9H_2O$ added to the rumen. A: sulphide concentration in the rumen after addition of $Na_2S.9H_2O$ equivalent to 113 μg. S/ml.
B: sulphide concentration in the rumen after addition of $Na_2S.9H_2O$ equivalent to 37 μg. S/ml.
C: increase in the blood sulphide concentration corresponding to the ruminal sulphide concentration shown in curve A.
D: Initial sulphide concentration (about 18 μg. S/ml.) taken as indicating a roughly constant sulphide level under normal ruminal conditions.

disappearance of sulphide from the rumen it is thought that most of the sulphide was absorbed through the ruminal epithelium, very little of it being utilized by the microflora.

Fate of Sulphate Added to the Rumen

The fate of sulphate in the rumen of the sheep was studied with three successive concentrations of $Na_2SO_4.10\ H_2O$, of 30, 60, and 90 g., respectively, equivalent to 3.0, 6.0, and 9.0 g. of sulphur. The sheep was found to have a rumen of the order of four litres. Samples of the ruminal contents were withdrawn at appropriate intervals and analysed for sulphide, sulphate, and protein sulphur, with the results shown in Fig. 4.

Blank experiment showed that in the absence of added sulphide or sulphate the level of sulphide concentration in the rumen remains fairly constant over periods of several hours, and was normally of the order of 15-30 μg. S/ml. The sulphide curves in Fig. 4b represent the increase in ruminal sulphide content arising from the added sulphate, being in fact the actual sulphide content less the initial pre-experimental content. The initial and final values of the pH of the rumen over the course of the experiments were always within the limits 6.0 to 7.0.

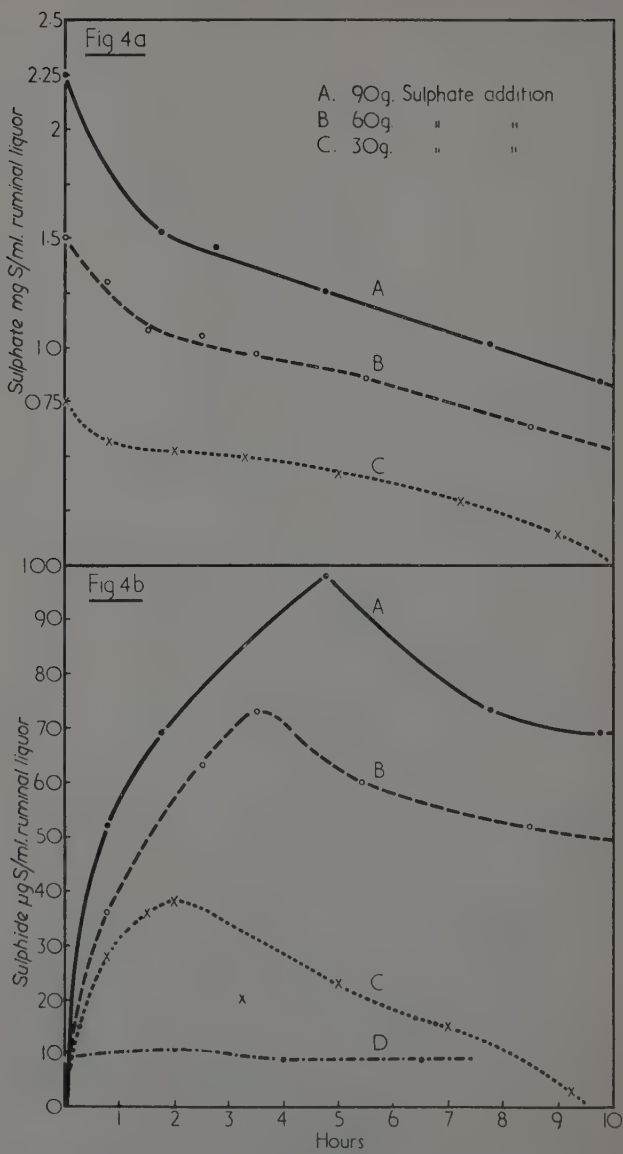


Fig. 4.—The fate of $Na_2SO_4.10H_2O$ added to the rumen. Fig. 4a: observed sulphate disappearance. Fig. 4b: observed sulphide production. Curve D is the observed ruminal sulphide concentration under blank-experiment conditions.

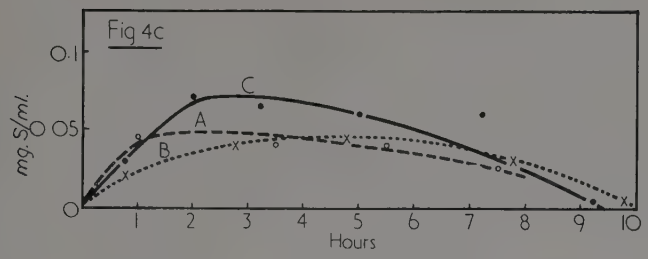


Fig. 4c.—Observed protein sulphur increase.
A: Crude-protein sulphur from the 90-g. sulphate addition.
B: Crude-protein sulphur from the 60-g. sulphate addition.
C: Crude-protein sulphur from the 30-g. sulphate addition.

Considering the sulphate disappearance curves and the observed sulphide production curves, it can be seen that in the period before peak sulphide production there has been a rapid reduction of sulphate to sulphide with a consequent build up of sulphide concentration, the rate of sulphide production having been greater than the rate of sulphide absorption or disappearance.

During the period after peak sulphide production, most of the sulphate has been used up or else the rate of production of sulphide has slowed down. In any case it is less than the rate of absorption of sulphide and the result is a steady decrease in the concentration of ruminal sulphide.

At the peak sulphide concentrations, the rate of sulphate disappearance (sulphide production) must be equal to the rate of sulphide absorption by the ruminal epithelium plus the rate of utilization of sulphide by the ruminal microflora. Unfortunately, the rates of sulphide disappearance, at these peak sulphide concentrations, cannot be obtained accurately enough from the curves of Fig. 3 to be equated to the equivalent rates of sulphate disappearance, save in the case of the 30-g. sulphate additions where, at the peak sulphide production of 38 μg. S/ml. the rates of sulphate disappearance and of sulphide absorption and bacterial utilization are both within the limits of 20-25 μg. S/ml./hour. In all events, sulphide absorption and bacterial utilization are very fast and prevent a very big build up of sulphide concentration in the rumen. This is evidenced by the peak sulphide production of 97 μg. S/ml. obtained during the experiment when sodium sulphate, equivalent to 9 g. sulphur (2,250 μg. S/ml.), was added to the rumen.

The curves derived from the crude-protein-sulphur fractions obtained during the course of the above sulphate studies are shown as curves relative to that obtained from the blank experiment, which was used as a base-line.

The initial crude-protein-sulphur values, which were variable, being 0.11, 0.09, and 0.18 mg. S/ml. respectively for the 90, 60, and 30 g. sodium sulphate additions, were in each case subtracted from the experimental values. In blank experiments these initial concentrations remained fairly steady over the period of up to 10 hours. The curves in Fig. 4c therefore show actual increases in the crude-protein content

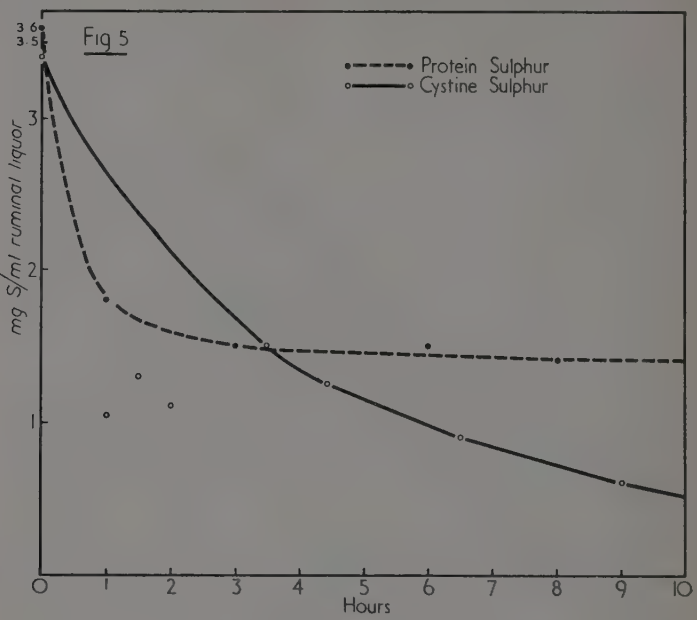


Fig. 5.—The fate of casein and cystine added to the rumen.

of the rumen. Some of the sulphide obtained from the sulphate reduction appears to have been used in the synthesis of microbial protein.

The "free-amino-acid sulphur" fractions were also determined, but these showed no significant variation and were always less than 0.04 mg./ml. of ruminal liquor.

Fate of Casein and Cystine Added to Rumen

In two separate experiments, 100 g. of casein, partly in suspension and partly in solution in 500 ml. of distilled water, and 5 g. of cysteine, also partly in suspension, were added to the ruminal contents in order to study the fate of the sulphur contained in these two compounds. By calculation, 100 g. casein contain 1.04 g. sulphur and 5 g. cystine contain 1.34 g. sulphur. The pH values for these two experiments, at the time of initial sampling, three hours later, and at the final sampling were, for the casein addition 5.9, 6.2, and 6.6, and for the cystine addition were 6.00, 6.24, and 6.43. In neither experiment was there any observed increase in the ruminal sulphide concentration, which remained at 25 μg. S/ml. and 15 μg. S/ml. for the casein and cystine additions respectively.

The curves shown in Fig. 5 for the recovery of protein sulphur and cystine sulphur have little meaning. The explanation is that much of the casein and cystine added to the rumen were in the insoluble suspended forms, a great deal of which was retained on the muslin upon filtering

the crude ruminal contents, and, consequently, the values obtained for protein sulphur and cystine sulphur were inaccurate. Qualitatively, the curves indicate that the amounts of casein sulphur and cystine sulphur in the rumen decreased during the courses of the two experiments.

Incubation of Sulphide with Liver Slices

To determine the fate of sulphide absorbed into the blood-stream from the rumen, tissue slices of liver and kidney from a freshly killed sheep were incubated with the not unreasonable amount of 50 μg. sulphide in Ringer's solution in Warburg vessels, aerobically at 37°C. Fig. 6 shows the results of two incubations of sulphide with liver and

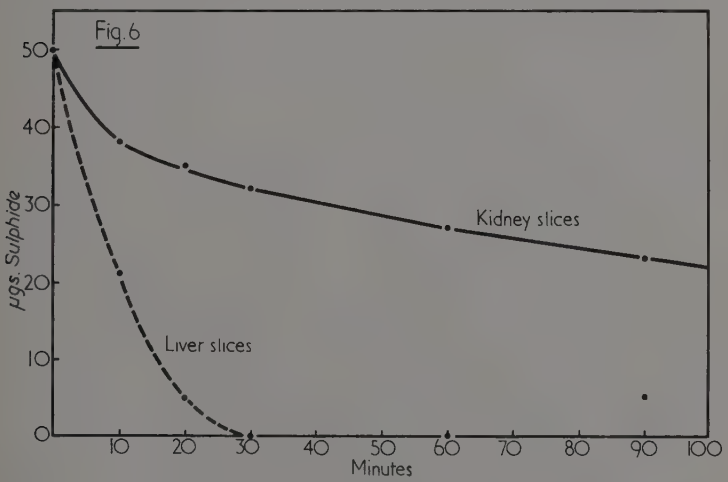


Fig. 6.—The incubation of sulphide with tissue slices.

kidney slices. The curves show a rapid uptake of sulphide by the liver slices, all of it having disappeared in thirty minutes, whilst the kidney slices also removed sulphide, but at a much slower rate. From the figure, and at the initial concentration of sulphide used, the liver slices removed sulphide at the rate of 96 μg. S/hour, using slices of average wet weight 0.1 g. Live sheep have a liver weighing approximately 600 g. and it is calculated, on the basis of utilization by slices, that a whole intact liver could take up about 0.6 g. sulphide per hour at the concentration used. This amount of sulphide is of the same order as that being absorbed from the rumen when the concentration of sulphide in the rumen is 130 μg. S/ml. (Fig. 3), the latter being higher than is likely ever to be achieved under normal conditions. This is confirmed by the absence of large increases in sulphide levels of blood draining from the liver. One would conclude that the liver can adequately cope with any sulphide released into the bloodstream from the rumen.

Thiocyanate Production in the Rumen

As a point of interest, it was decided to incubate solutions of cyanide and sulphide together with washed suspensions of ruminal microflora,

in Warburg vessels, to determine whether any thiocyanate would be produced as a detoxication measure. Coop and Blakley (1949) pointed out that ruminal fluid can slowly detoxicate HCN and considered this to be due to the H_2S and other sulphur donors present.

The method of Aldridge (1945) was used for estimating thiocyanate after deproteinization of the washed suspension by colloidal iron oxide, and it was found that washed suspensions containing 25 μg. CN′/ml. and 25 μg S″/ml., produced 4.5 μg. CNS′/ml. after 60 min., and 7.0 μg. CNS′/ml. after 90 min. Therefore cyanide can be detoxicated to thiocyanate by the ruminal microflora in the presence of sulphide.

Cyanide-induced Sulphur Deficiency in Sheep

Sulphur deficiency in sheep leads to loss of body weight and reduced rate of wool growth. Since cyanide is detoxicated to thiocyanate it is conceivable that under conditions of high cyanide intake a sulphur-adequate diet could be rendered sulphur-deficient.

An unsuccessful attempt was made to confirm this. Four individually fed sheep were paired off, weighed regularly, and had a 10-cm.-square patch of skin clipped every two weeks as a measure of wool growth. For the first 6 weeks the ration consisted of 680 g. sheep nuts containing 0.19% sulphur (= 1.3 g. daily intake of sulphur). One of each pair acted as a control whilst the other was dosed several times daily with potassium cyanide solution to give a daily intake of 0.4 g. cyanide ion. This should reduce the effective sulphur daily intake of the cyanide pair to 0.8 g. This treatment was without effect on the live weight or clean scoured weights of the wool produced. At the end of the six-week period the sheep were interchanged, the diet was changed to 680 g. of oat-straw chaff containing 0.11% sulphur (= 0.75 g. sulphur) plus urea as supplementary nitrogen. The experiment was continued for another six weeks. It was soon found however that on this lower-sulphur diet it was impossible to give daily the 0.4 g. cyanide ion without severe distress to the sheep. The cyanide intake had to be reduced steadily to 0.1 g. daily, the sheep becoming sensitive to even very small amounts of cyanide. The live weights and clean-scoured-wool weights again showed no differences. In the event, therefore, it was found impossible to reduce the sulphur content very much and to a level which could be considered deficient.

In New Zealand, high cyanide intake results from the ingestion of white clover. Clovers generally are high in sulphur and pastures containing a predominance of white clover would almost invariably have been topdressed with superphosphate, so that cyanide-induced sulphur deficiency would be most unlikely to occur.

Discussion

The reduction of sulphate to sulphide by the ruminal micro-organisms has been shown to occur most rapidly at pH 6.5, and the release of sulphide from protein and the uptake of sulphide at 6.0. These pHs are within the normal limits of the rumen.

The release of sulphide from a protein substrate (casein) and the utilization of sulphide by ruminal micro-organisms has been demonstrated *in vitro*. So also has the synthesis of new microbial protein

by micro-organisms utilizing sulphide obtained from sulphate reduction. Further, the rapid disappearance of sulphide by simple absorption from the rumen and the rapid uptake of sulphide by the liver (*in vitro*) has been shown. These points were briefly discussed during the presentation of the results.

Lewis has discussed the similarities of the sulphate-reducing bacteria in the rumen to the free-living organism *D. desulphuricans*. If these ruminal anaerobic bacteria do behave in a similar fashion, then sulphate is used as a source of oxidative power for the bacterial metabolic processes, sulphide merely being produced as an end-product, which may be used by other bacteria. The release of sulphide from protein, which is simply a splitting off of sulphydryl groups, may be brought about by bacteria different in type from the sulphate-reducing bacteria. Neither of these two types has yet been isolated from the rumen and studied in pure cultures.

Sometimes the studies *in vitro* with the washed suspensions gave inconsistent and low results; these may have been due to damage caused to the bacterial population. More precise information about the fate of sulphur in the rumen might be yielded by studies with compounds containing labelled sulphur and by studies on pure cultures of the micro-organisms.

On the basis of the above data, obtained both *in vivo* and *in vitro*, a provisional outline of the main events concerned in the metabolism of sulphur in the rumen may be given as follows: Food eaten by the animal arrives in the rumen and is immediately attacked by the micro-organisms present. Inorganic sulphate is reduced to sulphide which is shared, in varying proportions, by the animal host and the micro-organisms, the latter using their share to synthesize the sulphur-containing amino acids, which will be built into new microbial protein, whilst the rest of the sulphide is absorbed through the ruminal epithelium, passing into the blood to be taken up by the liver. When there is a large amount of sulphate present in the food, there is a corresponding increase in sulphide production accompanied by an increased rate of sulphide absorption, the animal host therefore getting the larger share of sulphide. On the other hand, when the amount of sulphate is small, the amount of sulphide produced is correspondingly small and the rate of absorption is slower, with the result that the microflora while not utilizing as much sulphide, utilize it more efficiently. When the inorganic sulphate is present in small amounts or has been mostly used up, food protein is degraded with release of sulphide which is, consequently, either absorbed through the ruminal epithelium or utilized by the micro-organisms in the synthesis of new microbial protein. "Free-amino-acid sulphur" should also undergo the same fate, while any sulphide in the diet would be directly utilized. We have therefore a picture of sulphate being reduced to sulphide, and food protein and "free-amino-acid sulphur" being degraded with release of sulphide to a common pool, from which the animal obtains its share by absorption into the blood with subsequent utilization by the liver, and from which the microflora take sufficient sulphide to enable them to synthesize new microbial protein which eventually accrues to the animal host.

The picture is one of an equilibrium between the sulphur composition of the diet and the changing character of the ruminal bacterial

population, with sulphide playing the role of key metabolite and with sulphate probably being the main source of sulphide. This outline agrees with the findings of the several workers mentioned in the introduction to this paper. It is also very closely parallel to nitrogen metabolism in the rumen, where "useless" nitrogen as ammonia may be upgraded to bacterial protein, and useful food protein down-graded to ammonia according to circumstances. The parallel is carried further, in that both sulphide and ammonia are absorbed from the rumen, so removing them both rapidly from the scene. The absorbed ammonia is generally considered to be nutritionally useless. In the case of sulphide, certainly the liver can remove it very rapidly, but the end-products have not been studied and we do not know whether absorbed sulphide has any nutritional value.

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GROWTH OF PASTURE SPECIES

III. WHITE CLOVER (*Trifolium repens*), SUBTERRANEAN CLOVER (*T. subterraneum*) AND LOTUS MAJOR (*L. uliginosus*)

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Summary

The effects of temperature, defoliation, and shading on the pattern of vegetative development of young plants of white clover, subterranean clover, and Lotus major (*L. uliginosus*) were determined. Plants were grown at mean temperatures of 53°F. and 72°F. At each temperature some were grown in full light and others under shading. Some of the plants grown in full light were defoliated.

For plants in full light the rise in temperature from 53°F. to 72°F. increased the rate of development most with lotus and least with subterranean clover. This was judged by rate of tissue formation by the whole plant, initiation of new stems, stem elongation, and amount of tissue formed on an individual tiller.

Elongation of the stems of lotus commenced very soon after germination, but with white clover it did not occur until the seventh leaf was appearing on the primary stem, 45 to 65 days after germination. For these species, the rate of elongation of individual stems rose to approximately 0.5 cm. per day. There was little stem elongation by plants of subterranean clover except where shaded at 53°F.

Successive leaves appeared more rapidly on a stem of subterranean clover than on white clover or lotus. The leaves of subterranean clover were also larger and hence the amount of tissue formed on an individual stem was greater; at 72°F., in full light, it reached 8–9 mg. per day which is comparable with rates for ryegrass. Under the same conditions it reached 6 mg. per day with white clover. For lotus, tissue formation on an individual stem was never above 1.1 mg. per day.

Introduction

A detailed study of the pattern of growth of some of the more important legume species was undertaken following similar work with grass species[6, 7].

The species chosen differ considerably in ecological adaptation in New Zealand. White clover is generally the dominant legume in permanent pastures on fertile land. Subterranean clover is an annual used in the warmer and drier districts where its ability to thrive on infertile soils has been of value. Lotus major is a valuable species on much of the wetter North Island hill country. It is able to grow among secondary growth or in wet areas.

The pattern of growth of the pasture legumes is more complex than that of the grass species previously examined. In the vegative phase there is a clear distinction in form between the leaf blades and petiole, and the elongating stems may constitute a considerable proportion of the total shoot tissue.

In introductory studies such as these, it is desirable to make detailed measurements on numerous features of growth in order that their inter-relationships may be understood. With this information, selection of a limited number of features for subsequent detailed study can be made.

Experimental Methods

The experimental procedures and treatments used were similar to those described in the previous paper[1]. There were fifteen plants in each treatment, five pots with three plants each. In this experiment the mean high-temperature level was 72°F. and the mean low level 53°F. For defoliation of white and subterranean clover the blades and most of the petioles were cut off all leaves on which the blades had commenced to unfold. The actual growing point of each stem, which lay on the soil surface, was not cut off. This treatment, with defoliation down to approximately ½ in., resembled a hard grazing. With lotus, the meristematic tips of the elongating stems reach well above the soil surface and would generally be removed during a grazing. Accordingly, about 70% of both stem and leaf tissue was removed at defoliation.

It had been intended that shading at both temperatures should reduce light intensity to 30% of full light. Inadvertently, one layer of the covering material was left off the shading frame used at 53°F.,

TABLE 1. Number of Days from Emergence to Commencement of Final Treatments and to Harvest, and Number of Leaves on the Main Stem at These Times

Species	Temperature	Treatment	Initiation of Final Treatment		Second Defoliation	Final Harvest	
			Day	Leaf No.	Day	Day	Leaf No.
White Clover	72°F.	Full light, undefoliated	46	6.7	53	73	12.0
		„ „ defoliated	„	6.8		86	11.2
		Shaded, undefoliated	„	6.9		91	11.7
	53°F.	Full light, undefoliated	63	6.7	70	93	11.4
		„ „ defoliated	„	6.7		101	11.3
		Shaded, undefoliated	„	6.4		106	11.3
Subterranean Clover	72°F.	Full light, undefoliated	36	6.4	42	66	16.9
		„ „ defoliated	„	6.9		72	16.9
		Shaded, undefoliated	„	6.9		79	16.3
	53°F.	Full light, undefoliated	50	7.1	57	98	16.4
		„ „ defoliated	„	7.5		99	16.2
		Shaded, undefoliated	„	7.3		108	16.9
Lotus	72°F.	Full light, undefoliated	44	6.9	56	71	12.5
		„ „ defoliated	„	6.7		77	7.9*
		Shaded, undefoliated	„	6.7		92	12.2
	53°F.	Full light, undefoliated	64	6.3	—	109	12.5
		„ „ defoliated	„	6.6		109	7.6*
		Shaded, undefoliated	„	6.4		112	12.5

* Number of leaves on dominant lateral stem

and these plants were therefore shaded to only 50% full light. Although this upset the balance of treatments the results were of considerable interest.

Table 1 states the number of days from the emergence of the seedlings to the commencement and termination of the treatments. Seedlings emerged on 13 July, and the temperature treatments commenced a few days later for all species. Defoliation and shading treatments were commenced in late August or early Sepember, depending on the rate of vegetative development of the species. The mean number of leaves present on the main stem when treatments were harvested differed between species but was the same at both temperature levels for each species.

In considering the results, conclusions based on statistically tested data are drawn only where the test used indicated significance at the 5% level of probability or better. For untested data, only major differences are noted. Standard errors of the mean are given in tables or indicated in diagrams where actual measurements are presented. Where results are derived by extrapolation or interpolation from actual measurements, co-efficients of variation are stated.

RESULTS

Growth of the Whole Plant

An estimate of the weight of shoot tissue formed in the 15-day period after final treatments commenced was on the assumption that increase in shoot weight was logarithmic (Table 2). With plants in full light and undefoliated, the quantity of tissue estimated to have been formed was greater at 72°F. than at 53°F. for all species. The relative difference was largest with lotus. Defoliation reduced the quantity of tissue formed by plants relatively more at 72°F. than at 53°F.

TABLE 2. ESTIMATED DRY WEIGHT (mg.) OF SHOOT TISSUE FORMED IN THE 15-DAY PERIOD AFTER TREATMENTS COMMENCED

Treatment	Mean Temperature	White Clover	Subterranean Clover	Lotus
		C	C	C
Full light, undefoliated	72°F.	232(28%)	269(12%)	21(47%)
	53°F.	175(42%)	191(14%)	31(50%)
Full, light, defoliated	72°F.	47(22%)*	76(19%)*	23(39%)*
	53°F.	53(67%)*	79(17%)*	7(42%)
Shaded, undefoliated	72°F.	46(33%)	89(14%)	28(31%)
	53°F.	127(48%)	169(21%)	35(55%)

Co-efficients of variation (in parentheses) are for the weights of plants at final harvest.

* Includes tissue removed after defoliation.

At 72°F., shading to 30% of full light gave a reduction in growth of 66% or greater. In contrast, the estimated reduction in growth at 53°F. from shading to 50% of full light was only 27% for white clover, 11% for subterranean clover, and nil for lotus.

During the earlier part of the experiment, the leaves of plants in full light at 53°F., particularly lotus, had discoloration suggestive of nutritional deficiency, although large quantities of balanced fertilizer had been applied when the seed was sown. When plants were transferred to the 50% shading, their colour rapidly changed to bright green and there was an initial appearance of faster overall growth by the shoot.

Variations in amount of shoot growth resulting from the different treatments were accompanied by changes in proportion of blade, petiole, and stem (Fig. 1). The differences between the species in the proportion of stem present reflect both differences in time when visible internode elongation occurred, and the rate of elongation of the stems. In lotus, internode elongation was present from the seedling stage, and these plants had a high proportion of stem. In white clover, visible internode elongation generally coincided with the appearance of the 7th leaf on the main stem. In subterranean clover it occurred at a still later stage of development and the internodes remained short.

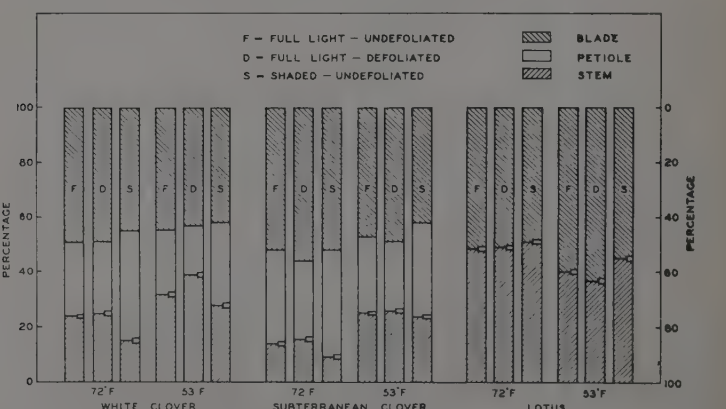

Fig. 1.—Proportion of blade, petiole, and stem tissue (dry wt.) in the shoot. Each group is (from left to right) full light, defoliated, shaded. Bars inserted indicate the S.E. of the mean for proportion of stem in each group of plants.

When plants were in full light, subterranean and white clover formed a higher proportion of stem tissue at 53°F. than at 72°F., but lotus formed more stem tissue at 72°F. At the higher temperature, shaded plants of subterranean and white clover were spindly with long petioles and little development of elongating stems, hence they had a low proportion of stem tissue.

Roots and Nodulation

The effects of treatments on proportions of root to shoot are set out in Table 3. The responses were similar to those found with grass

species[6, 7]. At 72°F. the proportion of root was reduced considerably by shading, but less by defoliation (for defoliation p = 0.30 for white clover, 0.05 for subterranean clover, and 0.10 for lotus). At 53°F., shading to 50% full light gave a considerable decrease in proportion of root tissue; but defoliation treatment apparently increased the proportion of root in white and subterranean clover, and made no difference at harvest in lotus.

TABLE 3. RATIO OF DRY WEIGHT OF ROOT TO DRY WEIGHT OF SHOOT

Treatment	Temperature	White Clover	Subterranean Clover	Lotus
Full light, undefoliated	72°F.	0.37 ± 0.06	0.60 ± 0.03	0.28 ± 0.04
	53°F.	0.45 ± 0.0[illegible]	0.58 ± 0.03	0.76 ± 0.16
Full light, defoliated	72°F.	0.29 ± 0.05	0.43 ± 0.08	0.20 ± 0.03
	53°F.	0.60 ± 0.09	0.77 ± 0.07	0.76 ± 0.06
Shaded, undefoliated	72°F.	0.14 ± 0.06	0.26 ± 0.01	0.10 ± 0.02
	53°F.	0.25 ± 0.02	0.26 ± 0.02	0.53 ± 0.04

Standard errors are for the means of five pots.

When the roots were being separated from the soil, marked differences in nodule development, due to treatments, were noted in all species. In general, the proportion of nodule tissue was reduced by higher temperature, by shading, and by defoliation, and the effects were cumulative. On undefoliated plants in full light at 53°F., there were numerous large convoluted pink nodules. At the other extreme, on shaded plants at 72°F., nodules were fewer in number, small, round, and white (Fig. 2). On defoliated plants there was a zone one third to half way down the root system where nodule development was poorer than either above or below. Presumably this was where nodulation was occurring at the time when the leaves were removed. The above observations are in accord with results reported by Wilson[10].

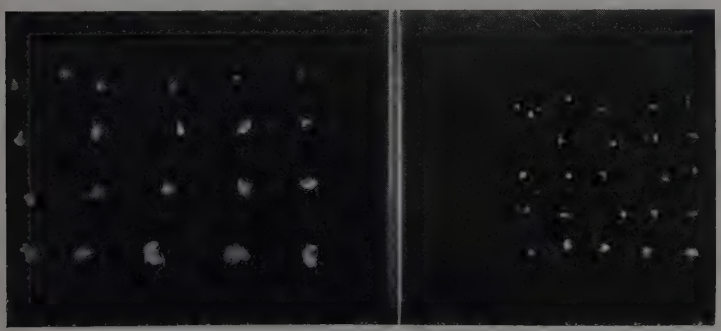

Fig. 2.—Nodules from roots of representative plants of subterranean clover grown in full light at 53°F.—undefoliated (left) defoliated (right).

Stem Development

Irrespective of where it is formed on the shoot, each active stem apex is a centre for formation of leaves and, potentially, for development of stolons or rhizomes. Figure 3 shows the rate of increase in stem numbers for plants in the various treatments, and Table 4 the number of stems present on the 65th day after emergence for undefoliated plants in full light.

The much slower rate of stem formation by the clovers, compared with the tillering of plants of perennial ryegrass, is apparent[5, 6].

At 72°F., the rate of stem initiation by undefoliated plants in full light was greatest with lotus and least with subterranean clover. A decrease in temperature to 53°F., without reduction of light intensity,

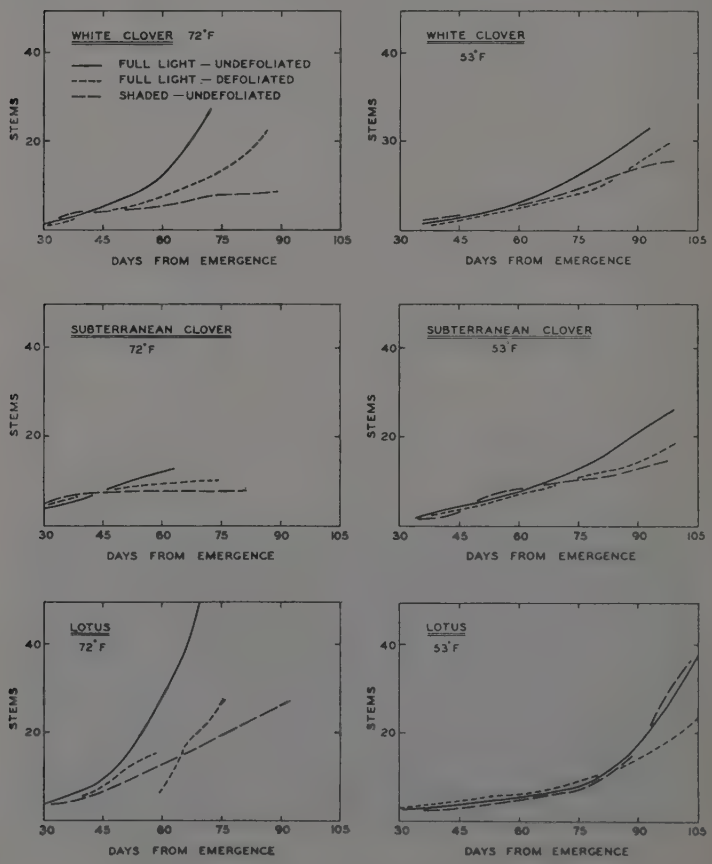

Fig. 3.—Daily increase in number of stems per plant.

TABLE 4. STEM NUMBERS AT 65TH DAY FROM EMERGENCE FOR PLANTS IN FULL LIGHT

Temperature	White Clover	Subterranean Clover	Lotus
	C	C	C
72°F.	17.7 (31%)	12.8 (7%)	36.5 (22%)
53°F.	5.8 (21%)	9.5 (17%)	6.3 (37%)

Co-efficients of variation are for stem number at final harvest

most severely reduced rate of stem initiation by lotus, and had least effect on subterranean clover.

As with grasses previously examined[6, 7], the relative reduction in rate of stem initiation resulting from defoliation was less at a lower temperature. As a result, after defoliation the rate of stem initiation by the clovers was of the same order at both temperature levels, and with subterranean clover even appeared to be faster at the lower temperature.

Shading to 30% light at 72°F. checked stem initiation severely. At 53°F., the shading to 50% light caused little reduction in stem initiation with lotus, but reduced it with the other two clovers.

These differences arose from considerable differences in the pattern of stem initiation in the three legume species. Lotus grown in full light formed not only axillary branches from the successive nodes along the enlongating stems but also initiated, every few days, fresh stems from the thickened crown that developed at the cotyledonary node.

In neither white nor subterranean clover did the main stem elongate, as it did in lotus. Internode elongation occurs initially with stems developing from the axils of the basal leaves on the main stem. In this experiment, subterranean clover showed only limited internode elongation and also relatively little development of second- and third-order stems. The plants generally formed a tight rosette only a few inches across.

In white clover there was no growth of adventitious shoots from a nodal crown, but there was usually extensive elongation of stems arising from the axils of leaves on the main stem. Under favourable growing conditions there was relatively little inhibition of lateral bud development either on elongating or non-elongating stems. Even on the elongating stems, many of the second- or third-order shoots commenced internode elongation. Thus, an interlacing complex of stems could be rapidly built up around the original seedling.

If in contact with a moist soil surface, large sturdy roots were rapidly initiated from the nodes of the elongating stems of white clover. This rapid rooting was less apparent in lotus, and where roots did appear they were small.

The extent to which the plants spread over the ground around the original site of germination is indicated by the numbers of elongated stems of various sizes (Fig. 4) and the lengths of the longest stems on

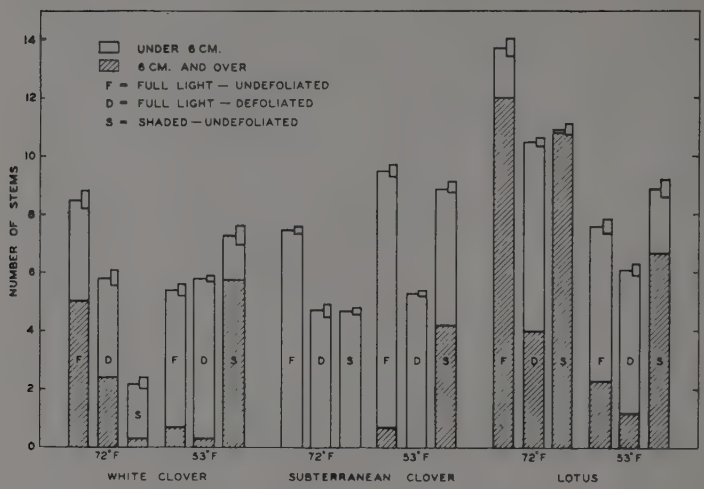

Fig. 4.—Average number of elongated stems per plant. Bars inserted at top of the colmuns indicate S.E. of the mean.

each plant (Table 5). For each species, plants were harvested in each treatment at the same stage of development, as judged by number of leaves on the main stem. This was approximately 12 leaves for both lotus and white clover, and 16 leaves for subterranean clover. It must be borne in mind that the age of the plants, in terms of the number of days from emergence, when these measurements were taken, varied (see Table 1) for all species and treatments.

TABLE 5. AVERAGE LENGTH OF LONGEST STEM ON EACH PLANT (cm.)

Treatment	Mean Temperature	White Clover	S.E.	Subterranean Clover	S.E.	Lotus	S.E.
Full light, undefoliated	72°F.	10.8	± 0.9	3.4	± 0.1	18.7	± 1.3
	53°F.	5.7	± 0.5	5.8	± 0.2	7.6	± 0.6
Full light, defoliated	72°F.	6.2	± 0.7	2.1	± 0.1	8.4	± 0.7
	53°F.	4.2	± 0.3	3.7	± 0.1	5.7	± 0.5
Shaded, undefoliated	72°F.	3.3	± 0.6	2.7	± 0.1	26.8	± 1.2
	53°F.	9.8	± 0.7	11.0	± 0.4	11.9	± 1.1

The standard errors are for the mean of fifteen plants.

The following points can be noted from the data.

(1) At 72°F., stem elongation, judged both by number of stems over 6 cm. and by length of the longest stem, was least in subterranean clover and greatest in lotus. At 53°F. relative differences between the species were less.

(2) Defoliation always caused a decrease in stem elongation.

(3) When shaded to 50% of full light, plants at 53°F. formed longer stems than when left in full light; shading to 30% of full light at 72°F. reduced stem elongation in white clover but limited it very little in lotus.

Particularly under conditions of low temperature or high light intensity, thickened whitish stems, with rudimentary leaves, appeared on plants of lotus (Table 6). These stems, which generally originated from the crown, grew down into the soil and could travel for several centimetres before re-appearing above ground. The existence of these stems in lotus species has been commented on by MacDonald[5].

TABLE 6. TYPES OF STEM ON LOTUS. MEAN NUMBER PER PLANT

Treatment	Temperature	Total Number at Harvest	Number Originating from "Crown"	Number of "Rhizome-type" Stems	
				Mean for Each Treatment	% of Plants where present
Full light	72°F.	52.2	11.6	2.9	100%
	53°F.	37.3	9.6	2.0	100%
Defoliated	72°F.	26.1	10.6	0.9	72%
	53°F.	23.4	9.7	0.6	53%
Shaded	72°F.	27.0	11.3	0.5	47%
	53°F.	35.1	9.7	2.5	86%

Dry Weight of Stem per Unit Length

This is considered an index of thickness of the elongated stems. It is greater in white clover than in lotus (Table 7). Elongated stems of subterranean clover were thicker still, but data were not recorded because on many plants there were no such stems.

TABLE 7. DRY WEIGHT PER CENTIMETRE OF STEMS (mg.)

Treatment	Temperature	White Clover	Lotus	
			Main Stem	Longest Stem
Full light, undefoliated	72°F.	4.9	0.9	1.0
	53°F.	6.6	1.5	1.6
Full light, defoliated	72°F.	5.0	0.8	1.0
	53°F.	5.3	1.0	1.0
Shaded, undefoliated	72°F.	4.7	0.3	0.6
	53°F.	6.5	1.1	1.5

In undefoliated plants of both white clover and lotus, stem weight per unit length was greater at 53°F. than at 72°F. It was reduced by defoliation of both species at 53°F. and, in lotus only, by shading at 72°F. The effect of other treatments on stem thickness was small.

Rate of Appearance of Successive Leaves on an Individual Stem

This was recorded on each plant in each treatment for both the main stem and an elongating axillary stem. As the rate was found to be similar for the two types of stems, results are presented only for the main stem. Fig. 5 shows the mean number of leaves formed on the main stem in the 21 days after final treatments commenced.

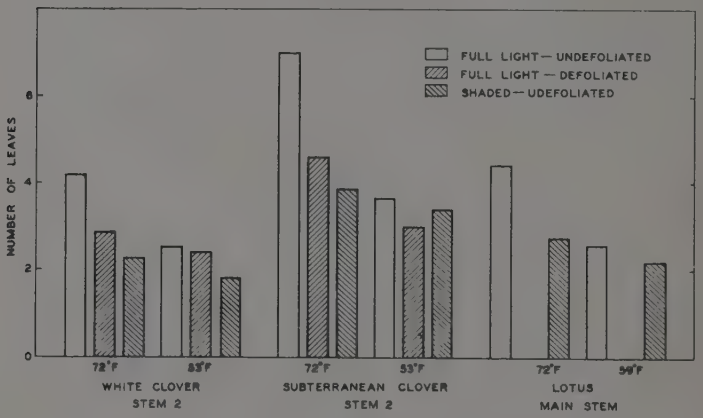

Fig. 5.—Mean number of leaves appearing on the main stem in a period of 21 days after treatment differences commenced.

As occurred with the grasses[6], either defoliation or shading tended to reduce the rate of appearance of leaves. In all treatments, leaves appeared in more rapid succession on subterranean clover than on the other species under similar treatment. At 72°F. in full light, undefoliated plants of subterranean clover formed more than two leaves per week, compared with 1 to 1½ leaves per week for the other species.

Leaf Dimensions

There was considerable variation in the size of the leaves, depending primarily on their position on the stem. Leaves were smaller on stems that were newly initiated, or were not elongating their internodes, than on vigorously elongating stems. A representative picture of the differences in leaf dimensions as between species and treatments comes from a comparison of the largest leaves on vigorously growing axillary stems (Table 8, Fig. 6).

As judged from these data, the leaves of lotus were, in general, only one-seventh the weight of those of subterranean clover and less than a quarter that of white clover grown under similar conditions.

TABLE 8. DIMENSIONS OF LARGEST BLADE AND LONGEST PETIOLE ON THE MAJOR AXILLARY STEM FROM EACH PLANT

Treatment	Temperature	White Clover—Stem from Axil of Leaf Two			Sub. Clover—Stem from Axil of Leaf One			Lotus—Longest Stem	
		Blade		Petiole Length (cm.)	Blade		Petiole Length (cm.)	Blade	
		Width (cm.)	Area (sq.cm.)		Width (cm.)	Area (sq.cm.)		Width (cm.)	Area (sq.cm.)
Full light, undefoliated	72°F.	3.1 ± 0.1	4.4	9.0 ± 0.5	2.9 ± 0.1	5.6	10.6 ± 0.3	1.9 ± 0.1	1.8
	53°F.	3.2 ± 0.1	4.8	7.7 ± 0.4	3.5 ± 0.1	8.3	11.9 ± 0.3	1.7 ± 0.1	1.6
Full light, defoliated	72°F.	2.7 ± 0.2	2.9	6.2 ± 0.4	2.3 ± 0.1	3.5	7.4 ± 0.2	1.4 ± 0.1	1.4
	53°F.	2.4 ± 0.1	2.3	5.0 ± 0.3	2.8 ± 0.1	5.0	7.7 ± 0.2	1.5 ± 0.1	1.4
Shaded, undefoliated	72°F.	2.7 ± 0.1	3.5	12.7 ± 0.6	2.8 ± 0.1	5.3	12.9 ± 0.2	1.9 ± 0.1	1.8
	53°F.	4.6 ± 0.2	9.9	12.3 ± 0.5	4.3 ± 0.1	12.2	19.4 ± 0.6	2.2 ± 0.1	2.3

Standard errors are for means of fifteen leaves

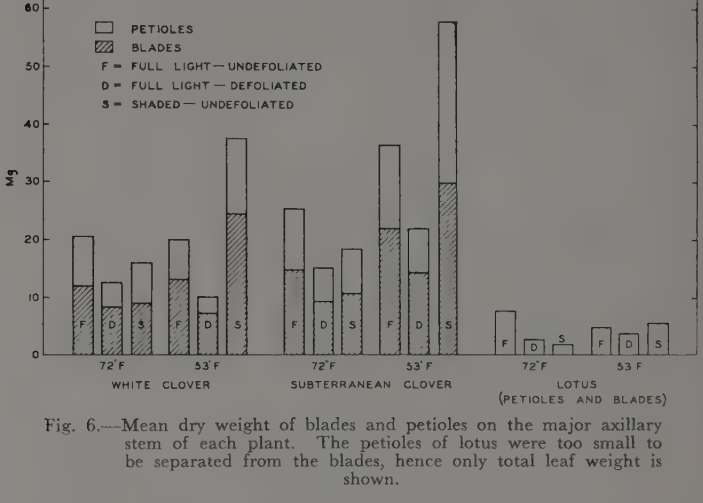

Fig. 6.—Mean dry weight of blades and petioles on the major axillary stem of each plant. The petioles of lotus were too small to be separated from the blades, hence only total leaf weight is shown.

In all cases defoliation reduced dimensions of subsequent leaves. Shading to 50% of the full light at 53°F. increased both petiole length and area of leaf blade, giving a considerable increase in weight per leaf. In contrast, leaves formed at 72°F. were of less weight on plants shaded to 30% of full light than on plants undefoliated and in full light.

Treatments influenced not only length of the petiole, but also its thickness, as judged by dry weight per cm. (Table 9). At 72°F., the shading treatment reduced petiole thickness, but at 53°F. increased it. Defoliation reduced petiole thickness. The petioles of subterranean clover were thicker than those of white clover, particularly at 53°F.

TABLE 9. DRY WEIGHT PER CENTIMETRE OF LEAF PETIOLES (mg.)

Treatment	Temperature	White Clover Stem 2	Subterranean Clover Stem 1
Full light, undefoliated	72°F.	0.95	1.04
	53°F.	0.89	1.31
Full light, defoliated	72°F.	0.69	0.81
	53°F.	0.56	1.03
Shaded, undefoliated	72°F.	0.55	0.59
	53°F.	1.01	1.45

There were also differences between treatments in pattern of development of the leaves on an axillary stem. When the leaf blade of white clover plants unfolded, shortly after growing out from the shoot apex, it had attained almost full size, but in subterranean clover enlargement of the blade continued for about one "leaf-appearance-interval" longer.

In subterranean and white clover there was a steady increase in size of successive mature leaves on a stem. It was greatest where plants were shaded.

The pinnate leaf of lotus reached full size very soon after appearance. The later-formed leaves on a stem tended to be progressively smaller.

In both these clover species the petiole, initially very short, elongated at a steady rate for several leaf-appearance intervals. On shaded plants, petiole elongation continued for a longer period but was also faster. This difference appeared immediately plants were shaded. On defoliated plants, petiole elongation not only ceased earlier than with plants in full light, but was also slower. For simplicity, only the data for typical plants at 53°F. are presented (Fig. 7). At 72°F., trends are the same but differences between treatments less.

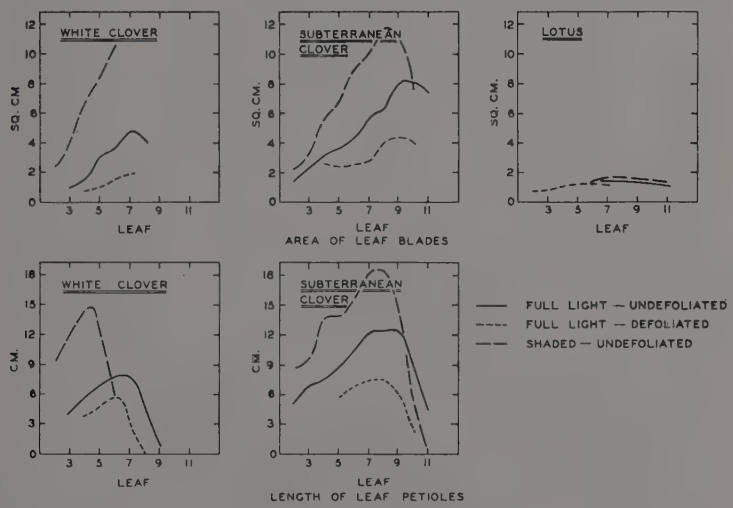

Fig. 7.—Dimensions of successive leaves on the major axillary stem of each plant.

Tissue Formation by a Single Stem

The simplicity of the course of tissue formation by a tiller of the grasses examined previously[6, 7] is absent in the clovers. Instead of linear-shaped leaves growing from basal intercalary meristems, there is, in these legumes, development of distinctively shaped leaf blades and leaf petioles, plus elongation of the true stem. The wave of growth occurs first in the leaf blades and then in the petioles and stem internodes. Its duration and speed in each tissue differed as between species and treatments. For petioles and internodes it extended over several plastochrone intervals. As a result, obtaining a day-to-day record of the course of tissue formation on a stem was more difficult than with a grass species, and the data are much more complex. In this paper these are summarized in two sets of results. Firstly, the rate of elongation

of selected stems of white clover and lotus is shown in Fig. 8, and secondly, the average amount of blade, petiole, and stem tissue formed per day in the five days before final harvest of the plants is shown in Fig. 9.

Stem elongation in lotus is initially greatest on the main stem but later the rate of growth of many of the lateral stems surpasses it. Detailed

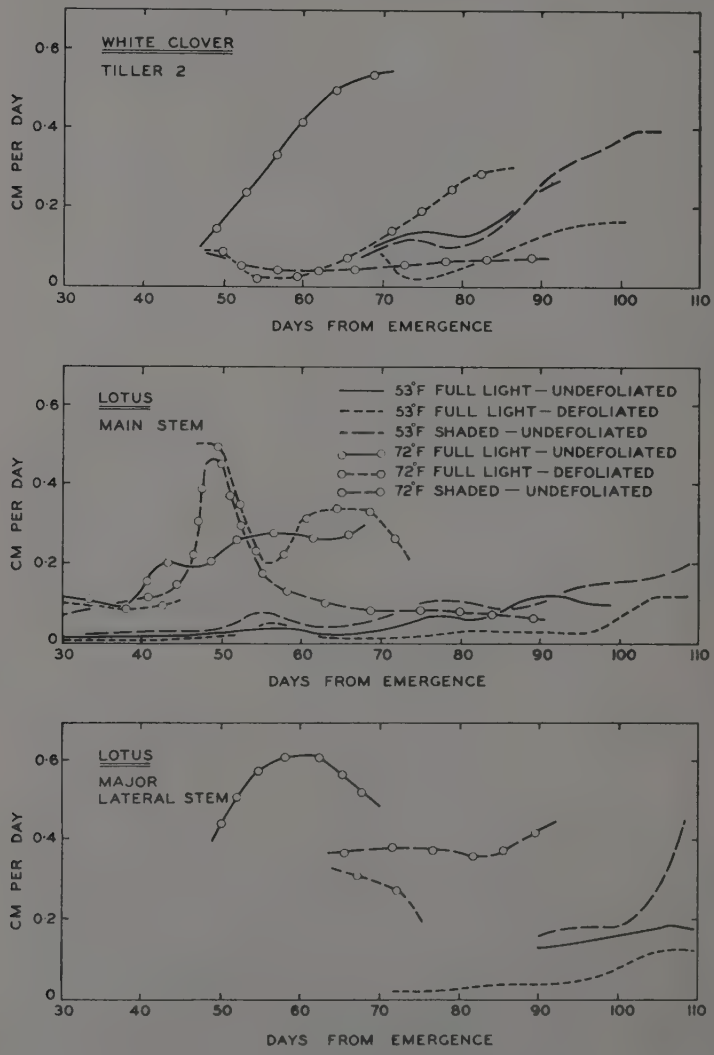

Fig. 8.—Daily increase in length of elongating stems.

measurements upon main and lateral stems are summarized in Fig. 8. In defoliation, the growing points of the largest stems of lotus were removed. In this case rate of elongation of the main regrowth stem was measured.

Owing to a general slower development of white clover at the lower temperature, stem elongation commenced later in the plants at 53°F. At both temperatures the stem arising from the axil of the second leaf on the main stem commenced elongating when the seventh leaf was appearing.

Defoliation always reduced rate of stem elongation but the reactions to shading were more complex. At 53°F., shading to 50% of full light did not lessen the rate in white clover, and appeared to stimulate it in lotus and also in subterranean clover (note Fig. 4, Table 5). At 72°F., shading to 30% full light very nearly halted stem elongation of white clover, but reduced it by only about a third on lateral stems of lotus. When lotus was shaded at 72°F., there was an immediate stimulation of stem elongation, but it was of short duration.

Where plants were at 72°F. in full light, there was little difference between lotus and white clover in the rate of stem elongation finally attained, about 0.5 cm. per day in intact plants, and about 0.25 cm. in defoliated plants. At 53°F., stems of white clover elongated faster than those of lotus.

Although, at the stage of development examined in these experiments, subterranean clover neither formed new stems rapidly nor gave much stem elongation, the individual stems that were present formed leaf tissue much faster than did individual stems of white clover or lotus (Fig. 9). At 72°F. in full light, a stem of subterranean clover formed

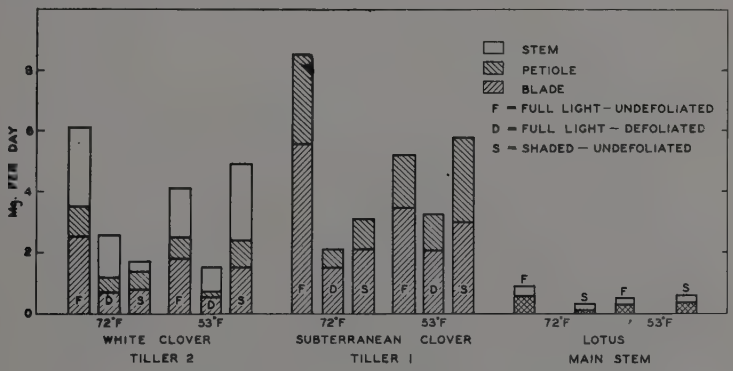

Fig. 9.—Average weight of tissue formed per day on the major axillary stem of each plant, during the five days prior to final harvest.

8.7 mg. of leaf tissue per day, compared with 3.5 mg. and 0.6 mg. of leaf tissue for white clover and lotus respectively. In all treatments the subterranean clover stem formed leaf tissue about twice as fast as the stem of white clover. The amount of tissue, including both stem and leaf, formed by an individual stem of lotus was not more than 1.1 mg. per day in any treatment.

Discussion

Although caution is required in using results from single-plant studies to predict the reaction of a species or strain in the field, certain deductions would appear admissible.

In lotus, a notable feature during the trial was the contrast between the rapid growth of plants given full light and the higher temperature, and their slow growth under the cooler conditions. At 53°F., the young plant was small and the actual weight of tissue formed in the 15-day period quoted was only about a sixth the quantity of tissue formed by subterranean clover. It is not surprising that lotus is considered a very slow-establishing plant in cooler districts, particularly from autumn sowings. The rapid growth required for it to spread aggressively in a pasture will occur only in periods of warm weather.

In a closely grazed pasture the habit of growth of lotus further militates against its spread. The plant has relatively small leaves set close to stems that often grow well above ground. Hence, grazing will remove not only the leaves, but also the stems with their relatively large apical meristems. As lotus plants form comparatively large numbers of stems, there would generally be a considerable number of active meristematic centres remaining after defoliation from grazing. However, these would be on small stems, for many of which active internode elongation would not have commenced. They would initially form less tissue than the large apical meristems removed.

This suggests that recovery from defoliation would be slower than in subterranean or white clover in which the meristematic centres of tissue formation at the shoot apices are not normally removed during grazing. Actually, in plants of lotus at 53°F., recovery from defoliation was so slow that the second defoliation was omitted in this experiment.

The tendency for the young plant of lotus to form a distinct crown set fairly well above the soil surface would also make it more susceptible to damage by close sheep grazing.

Conversely, there are several features of the growth of lotus that would enable plants to spread rapidly through a pasture, given appropriate temperature and light conditions. At 72°F., elongation of the numerous stems formed by an ungrazed plant would enable it to form a densely interwoven cover over the ground. Many of these stems lie close enough to the soil surface to escape easy removal during grazing. In addition, the plant will be forming a number of underground stems.

In a long pasture, or among fern and scrub, the ability of the stem itself to grow up among the vegetation should better enable leaves to be placed above dense shade from other vegetation, than where plants have to rely on petiole elongation alone. With severe shading at the higher temperature, the whole plant became rather spindly with a limited number of relatively thin but long stems. Under moderate shading at cooler temperatures, the plant appeared well adapted for competition in a relatively tall association. The aerial stems were robust, and elongated even faster than those of plants in full light.

The autecology of lotus is in contrast with that of subterranean clover, which is an annual relying for its spread through a pasture on the rapid elongation of flowering stems in the late spring, and subsequent

placement of the seed on or into the surface soil. During the remainder of its growing season, i.e., from seed germination in the autumn until the late spring, the plants shows relatively little stem elongation. In full light the plants grew in a rosette form with the rate of initiation of fresh stems, as centres of growth, remaining low. However, by forming from each stem relatively large leaves at a much faster rate than for the other legumes, they produced more leaf tissue from each individual stem. These rates of tissue formation compare favourably with those of a tiller of perennial ryegrass under similar conditions[6]. Whereas defoliation severely slowed down formation of leaf tissue at 72°F., it reduced it by only 36% at 53°F. It follows that subterranean clover should be a good source of winter feed under close defoliation.

The pattern of development of white clover was intermediate between those of the other two species. Formation of leaf tissue on an individual stem was faster than in lotus, but slower than in subterranean clover. However, rate of stem initiation was faster than in subterranean clover, but slower than in lotus. Consequently, white clover should show less tendency to form a dense cover of elongating stems over an area of ground than lotus. However, the elongating stems of white clover are more robust than those of lotus; they elongate faster at low temperature, grow close to the soil surface, where they should generally escape damage during defoliation, and readily root at each node. Except in tall growth, where inability to form sufficiently long petioles would cause excessive shading of the leaf blades, these features of the stem growth should enable the plant to compete well with other species under a wide range of pasture conditions.

In white clover, stem elongation commenced at both levels of temperature about the time of appearance of the seventh leaf on the main stem, i.e., 45 days after seedling emergence at 72°F., and 65 days at 53°F. for plants in full light. Winter cold, particularly if accompanied by heavy shading, as where a young pasture is allowed to grow long, would considerably extend that 65-day period for initiation of stem elongation by reducing rate of leaf appearance on the primary stem. Until stem elongation occurs, the growth of the plant from a single crown would make it susceptible to grazing and trampling damage, particularly if the plant is drawn up by shading. The need to keep pastures short during the establishment of clovers has been emphasized by Suckling[8] and Brougham[3].

TABLE 10. DIAMETER COVERED BY ELONGATING STEMS IN 14 DAYS (inches)

Treatment	Temperature	White Clover	Lotus
Full light, undefoliated	72°F.	5.8	5.8
	53°F.	4.1	2.0
Full light, defoliated	72°F.	3.2	2.6
	53°F.	1.5	1.3
Shaded, undefoliated	72°F.	0.8	4.3
	53°F.	4.3	3.3

Once stem elongation commences, clover plants should be able to spread through a sward quite rapidly. Table 10 sets out the diameter and area of ground which would be covered by stems of a single plant in a fortnight when elongating at the rate measured shortly before harvest. It is assumed that for each stem elongating in one direction, there is another elongating at the same rate in the opposite direction. With rooting occurring at many stem nodes, particularly in white clover, there will be, in effect, rapid increase in number of effective plant units and their spread through the pasture.

Table 10 also makes it clear that at higher temperatures the spread of white clover by stem growth will be very small if the plant is severely shaded. Under these conditions the plants in all respects appeared fragile. With the mean length of the longest petiole only 5 in., the plant is obviously not adapted to compete in pasture that is allowed to grow long under high temperatures.

Issues of at least equal importance are raised by the observations on relative root and nodule development. Although interactions between defoliation, light intensity, and temperature, on both relative root and nodule development cannot be understood until more is known of the mechanisms governing metabolite distribution within the plant, and the kinetics of growth at various meristematic centres, the question is of sufficient agronomic importance to justify comment. For instance, plants of white clover, and particularly of lotus, growing in full light under cool early spring conditions showed leaf coloration and death suggestive of nitrogen deficiency. Yet these same plants formed relatively large root systems which carried numerous large pink nodules. If light intensity was reduced by half, there was relatively less root tissue and the nodules were smaller and not so pink, but foliage was a lush green, characteristic of ample nitrogen status. The rate of growth of the shoot was reduced little by 50% shading, probably largely owing to the diversion of assimilate to the shoot rather than the root meristems. The additional leaf tissue formed by the shoot meristems would itself form more assimilate, and thus help to compensate for the reduction in rate of photosynthetic assimilation that probably accompanied the reduced light intensity.

All of the points brought out by the results, i.e., that moderate shading may check shoot growth only a little or not at all[2], that relative root growth is lowered by shading, defoliation, or temperature increase[6, 7], and that nodulation is checked by high temperatures or defoliation[1, 10] have been reported on a number of occasions. However, the extremes that can be reached have not been emphasized nor have their integrated agronomic implications been considered. It has often been suggested that, in pastures, plants should have a large root system, indicating adequate reserves[4], and large pink nodules, indicating vigorous activity by the symbiotic bacteria[9]. Under some conditions both may be desirable for pasture thrift, but there are probably other sets of environmental conditions, which may include a considerable portion of a New Zealand year, where the presence of numerous large pink nodules may not automatically result in a high effective nitrogen status in the tops and a large root system may be little benefit for growth of herbage.

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THE EFFECT OF PHENOTHIAZINE ON THYROID GLANDS OF SHEEP

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Summary

To examine under normal farming conditions the possible importance of the reported antithyroid effect of phenothiazine, thyroid glands from sheep given a total of 120 grammes of a commercial preparation of the drug in 6 doses over a period of 13 weeks, were compared with those from control animals receiving no phenothiazine. The mean wet weight of thyroid glands from phenothiazine-dosed sheep was slightly, though not significantly, less than the corresponding figure obtained for glands from control sheep. Presumably as a result of the iodine contained in phenothiazine, the mean percentage iodine content of glands from phenothiazine-dosed sheep was nearly twice as high as that for glands from control animals. It is concluded that phenothiazine does not exert a goitrogenic effect on the thyroid glands of sheep.

Introduction

Talmage and co-workers have shown that single doses of phenothiazine exert an antithyroid effect on rats (Talmage, Nachimson, Krantz, and Green, 1953), burros (Talmage, Trum, Monroe, and Rust, 1953), and other farm animals including sheep (Talmage, Monroe, and Comar, 1954), as measured by reductions in the rate of uptake of radio-iodine by thyroid glands. Antithyroid activity appeared to reside in impurities contained in samples of the phenothiazine used. Since iodine is employed as a catalyst in the manufacture of phenothiazine, it was suggested that excess iodine contained in the drug and liberated as iodide after ingestion could be responsible for the effects shown. However, in a later publication (Nachimson *et al.* 1954), it was established that, although the antithyroid activity was strongly associated with a fraction containing most of the iodine, when rats were fed phenothiazine no enlargement of the thyroid gland occurred and little or no iodine appeared in the urine. It was concluded that the effect of unpurified phenothiazine in reducing uptake of radio-iodine was not due solely to the availability of free iodide to the gland.

Because of the extensive use of phenothiazine for the control of internal parasites in farm animals, it seemed desirable to determine whether commercial preparations possess goitrogenic properties when given under conditions comparable to those obtaining in practice. The present paper describes the effects of regular phenothiazine dosing on the weight, dry matter content, and iodine content of the thyroid glands of sheep.

Experimental

Experimental Sheep

The animals used were Romney Southdown crossbred ewe hoggets approximately 8½ months old at the commencement of the experiment. For the duration of the trial they were confined to pastures consisting predominantly of perennial ryegrass and white clover. On 11 May

1954, they were weighed and divided as evenly as possible on a body-weight basis in the following two groups of 13 sheep.

Group 1	Control
Group 2	Phenothiazine-dosed

At the start of the experiment, group 2 animals each received orally a dose containing 20 g. of an aqueous suspension of a commonly-used commercial preparation of phenothiazine. Thereafter, three further doses were given at fortnightly intervals, followed by another two at three-weekly intervals, the last dose being given on 10 August. Group 2 animals therefore received a total of 120 g. phenothiazine in six doses over a period of 13 weeks. Three sheep in the control group died from parasitism. On 26 August the survivors were re-weighed and then slaughtered. The thyroid glands were removed, weighed immediately, and retained for iodine analyses.

Iodine Analyses

Thyroid Glands. The method used in this laboratory is based on that of Leitch and Henderson (1926) with modifications suggested by H. D. Purves (pers. comm.). Because the present procedure appears to offer some advantages in speed over similar methods previously described, it is given here in detail.

After weighing, the fresh glands are cut into small pieces and a sample of up to 2 g. weighed into tared, 50-ml. nickel crucibles. Dry matter is determined by drying in an oven at 105°C. for 48 hours and re-weighing. To each sample 10 ml. of 10% aqueous KOH solution are added, watch glasses are fitted to each crucible, and the samples are digested on a water bath for about 8 hours. A blank is added at this stage. The samples are next taken to dryness and charred. They are transferred to a muffle furnace and the temperature is raised over a period of about 2 hours to 450°C. Lids are fitted to each crucible and the temperature allowed to rise to 520°C. After removal from the furnace, distilled water is added to the cooled, partly-ashed contents of each crucible. The samples are well stirred and remaining carbon particles are filtered off under suction through a fine-meshed sinter-glass funnel to which is fitted a small disc of Whatman's No. 42 filter paper. Filtrates and washings are collected in 50-ml. volumetric flasks and made up to volume. Duplicate 5-ml. aliquots are taken from each flask and transferred to 50-ml. pyrex test tubes. Two drops of 0.5% aqueous solution of methyl orange are added and the solutions made faintly acid with approximately N/2 H_2SO_4. To oxidize iodide to iodate, bromine vapour is poured into each test tube to give a definite yellow colour when mixed. After a few minutes, excess bromine vapour is blown out, boiling sticks are added to each test tube and unused bromine boiled off. The final volume should be about 3 ml. The samples are cooled, and, to each, two or three crystals of KI are added. The liberated iodine is titrated with standard N/500 sodium thiosulphate solution to a colourless end-point using starch indicator. From the results, total iodine and iodine concentration in wet or dry thyroid glands can be calculated.

Phenothiazine. Since commercial preparations of phenothiazine would be expected to contain traces of iodine, it was of interest

to examine samples of the drug for iodine content. Because of difficulties in dry ashing phenothiazine, total iodine was not determined in the present work. It was, however, considered likely that boiling KOH would extract any inorganic iodine, and probably much, if not all, of less firmly-bound organic iodine. Accordingly 2-g. samples of phenothiazine powder were extracted with 10 ml. of boiling 10% KOH. Filtrates and washings were taken to dryness, ashed, and the iodine content determined in the manner described for thyroid glands.

TABLE 1. Effect of Phenothiazine on Body Weights and Thyroid Glands

Group	Body Weight Change (lb.)	Thyroid Glands:		
		Wet Wt. (g.)	Dry Matter (%)	Iodine (% D.M.)
1. Control	—1.1 ± 2.3*	2.9 ± 0.5*	31.3 ± 1.2*	0.300 ± 0.030*
2. Phenothiazine	+8.6 ± 1.2	2.7 ± 0.2	34.6 ± 1.0	0.542 ± 0.027
Differences:	9.7	0.2	3.3	0.242
Significant at:	1%	N.S.	5%	0.1%

* Standard errors of means.

TABLE 2. KOH-extractable Iodine in Phenothiazine Samples

Sample	Source	Iodine (%)
A	Australia	0.18
B	Australia	0.14
C	Britain	0.26
D	Britain	0.11
E	U.S.A.	0.19
F	Germany	0.13

Results

Table 1 gives mean body-weight changes of the two groups of sheep between 5 May and 26 August, and compares means for fresh weight, dry matter content, and iodine concentration expressed as percentage iodine on a dry-matter basis, for thyroid glands from each group.

In Table 2 is shown KOH-extractible iodine in a phenothiazine sample (A) representative of that dosed to group 2 sheep. Corresponding figures for samples from other sources (B, C, D, E, and F) are given for comparison. Results are in each case expressed as percentages of the dry powder.

Discussion

Results in Table 1 show that the mean body-weight gain made by phenothiazine-dosed sheep (group 2) was significantly greater than that for the control group (1). The beneficial effect of phenothiazine was presumably due to its anthelmintic activity.

Perusal of the literature (e.g. Mason, 1933; Sykes, 1934; Sinclair and Andrews, 1954) suggests that, for sheep less than 12 months old, weights of normally-functioning thyroid glands are usually of the order of 2 g. and seldom exceed 5 g. In the present trial two sheep in group 1 had glands weighing slightly more than 5 g., whereas for group 2 all glands weighed less than this amount. Means for both groups were within normal limits and the small difference between them was not significant.

In all thyroids from both groups the iodine concentrations were considerably in excess of the figure of 0.10% on a dry-matter basis considered by Marine and Lenhardt (quoted by Waters, 1938) as the "critical level", or minimal percentage of iodine required for normal functioning of the gland. The markedly higher mean iodine content of glands from group 2 as compared with that for group 1 is discussed later.

The physiological importance of the significantly lower value for the mean dry-matter content of the glands from the undosed group (1) is not known but could have been due to slight oedema resulting from parasitic anaemia.

Table 2 shows that 6 samples of commercial phenothiazine from various sources all contained appreciable amounts of iodine. From the results found for Sample A (0.18% I) it can be calculated that with each dose sheep received at least 36 mg. iodine or a total of 216 mg. Working with rats Nachimson *et al.* (1954) found that doses of 200 mg. of phenothiazine found to contain 0.4% of what was termed "organically-bound" iodine failed to increase the output of urinary iodides. From this they concluded that "iodine in phenothiazine is not immediately released and consequently is not available in circulation as free iodide ion". On the other hand our results show that the iodine content of thyroid glands from group 2 sheep was markedly increased as a result of phenothiazine dosing (Table 1), and it is reasonable to suppose that this was due to the extra iodine received with the drug. So far as is known the thyroid gland does not accumulate appreciable amounts of iodine in any form other than as iodide ions. Present results suggest, therefore, that the iodine in phenothiazine is available to the thyroid glands of sheep as inorganic iodide.

Since regular doses of phenothiazine failed to increase the size of the glands and enhanced rather than diminished their iodine content, it is concluded that commercial preparations of the drug do not exert a goitrogenic effect on the thyroid glands of sheep.

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PEAT ON MOKAI PATEA, RUAHINE RANGE, NORTH ISLAND, NEW ZEALAND

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(Received for publication, 16 September 1955)

Summary

Peats at altitudes of between 4,000 and 5,000 ft. on Mokai Patea, part of the Ruahine Range, are described, and the manner of their formation is discussed. Peat is still accumulating on two of the mires discussed and on these two there are numerous tarns; but, on mires where accumulation has ceased, the peat is either being destroyed by erosion, or has been overgrown by tussock vegetation. The data presented suggest that peat formation formerly has been more active on Mokai Patea than at present, and that those mires on which peat is still accumulating are largely dependent on seepage water from tarns for their continued maintenance.

Introduction

Over the last twenty years, papers describing certain lowland peats have been published (Cranwell 1939, Harris and Filmer 1946, Harris 1951, Moar 1953, 1954) but there have been none describing mountain peats in New Zealand. Cranwell (1953) has, however, discussed in some detail a peat profile from Swampy Hill (2,433 ft.) near Dunedin. In February 1951, mires at altitudes above 4,000 ft. on Mokai Patea were investigated, and it is the purpose of this paper to describe these and to discuss their formation.

Mokai Patea is a north westerly extension of the central Ruahine Range and it is separated from the main axis by a low forest-covered saddle. Rongotea, the highest peak, rises to 5,144 ft., and two lesser peaks, Mokai Patea and Colenso, rise to 3,969 and 4,813 ft. respectively (Fig. 1). From the saddle between Colenso and Rongotea (about 4,000 ft.) the main ridge at first rises steeply, but soon flattens and becomes broad and undulating, rising gradually to Rongotea.

The peats described (only a few of many mires were examined) lie on this broad undulating surface as a series of small and scattered accumulations, the largest of which is not more than two or three acres in area. They generally lie in wide shallow drainage channels, but some of the smallest have accumulated on steep slopes. Peat is accumulating in some mires, and, on these, tarns are a characteristic feature; but others have ceased to grow and are now being eroded by wind and rain, or have been overgrown by tussock vegetation. In some cases erosion may have been initiated by trampling deer. Borings were made on three mires. The peat in these, in which traces of volcanic ash were found, was shallow and fibrous and overlay clay and rock.

Climatic records are not available for Mokai Patea, the nearest recording station being at Taihape (1,450 ft.) about 20 miles to the west. The range has an annual rainfall of at least 60 inches (Garnier, 1950), and is subject to frequent mists and to winter snows.

Vegetation

Although a similar pattern of vegetation occurred in each mire, there were differences that could be related to topography, erosion, or cessation of peat accumulation.

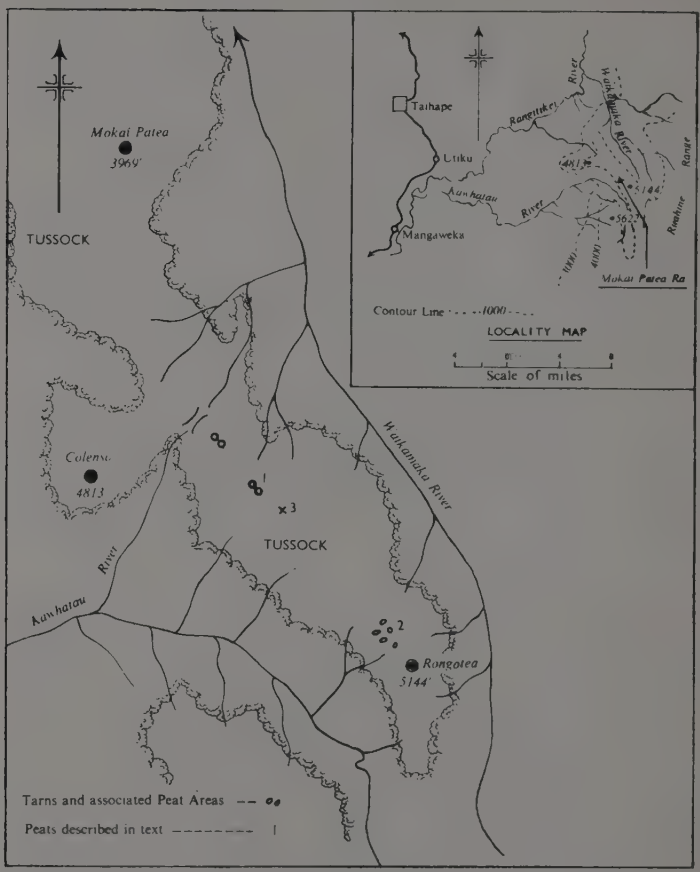

Fig. 1.—Sketch map of Mokai Patea ridge, a north-west extension of the Ruahine Range.

Most tarns were surrounded by a bank of living *Sphagnum*, and, where the ground fell steeply from the tarn, this plant extended for some distance down-slope. Growing through the *Sphagnum* and rooted in underlying layers were *Schoenus pauciflorus*, *Danthonia flavescens*, and, sometimes, *Dracophyllum recurvum*; while at the tarn edge *Sphagnum* was usually the only plant present. There were several species rooted in the surface layers of the *Sphagnum*, including *Juncus novae-zealandiae*, *Nertera depressa*, *Plantago uniflora*, *Euphrasia revoluta*, *Forstera bidwillii*, *Gentiana bellidifolia*, *Celmisia gracilenta*, *C. glandulosa*, *Pentachondra pumila*, and *Astelia linearis* var. *novae-zealandiae*.

On steep slopes subject to seepage from tarns or from higher ground, and on the gently sloping surface of the larger mires, there were extensive cushions of *Oreobolus pectinatus* through which *Carpha alpina, Schoenus pauciflorus,* and, occasionally, *Danthonia flavescens* were growing, the two latter species being rooted in the underlying peat. Other plants common to these areas were *Astelia linearis* var. *novae-zelandiae, Plantago uniflora, Celmisia gracilenta,* and *Gentiana bellidifolia.*

On flat, sopping-wet surfaces there were mats of *Scirpus aucklandicus, Gaimardia pallida,* and *Juncus antarcticus,* in which such species as *Plantago uniflora, Epilobium pernitens, Hydrocotyle novae-zelandiae* var. *montana,* and *Carex stellulata* were common.

Vegetation dominated by *Danthonia flavescens* and not readily distinguishable from tussock vegetation of non-peaty areas is growing on peats that have ceased accumulating and have not been destroyed by erosion. It is only when the soil surface is broken that peat is revealed. In one such area *Sphagnum* peat was found beneath a dense vegetation of *Danthonia flavescens, Dracophyllum recurvum,* and *Schoenus pauciflorus.*

The vegetation of eroding surfaces varied from place to place. Scattered cushions of *Carpha alpina* and of *Oreobolus pectinatus* were noted, and on the bed of a destroyed tarn a mat of *Gaimardia pallida* was recorded, this species also occurring on the bottom of existing tarns. On a valley side, *Sphagnum* was growing in small mounds in wet depressions on eroding *Schoenus* peat.

PEAT AND PEAT PROFILES

As borings were made on three fairly widely separated mires each will be discussed separately (Fig. 1).

Mire 1

Two borings were made: the first near the edge of a tarn, and the second half a chain down slope from the first and near the edge of a second smaller tarn where cushion plants were dominant.

In the first profile, the upper layers of peat had been formed by *Sphagnum* that was not much decomposed, the deeper layers being composed mainly of monocotyledonous fragments, many of which were the bases of *Schoenus pauciflorus.* A description of this bore-hole is recorded in detail from field notes:

Depth (inches)	
0 – 24	*Sphagnum* with bases and rhizomes of *Schoenus pauciflorus* *W4, *H3
24 – 30	An abrupt transition to slightly fibrous peaty loam; H7;
30 – 36	peaty loam; some *Schoenus pauciflorus* bases; H8;
36 – 37	black volcanic ash;
37 – 42	peaty loam; monocotyledonous remains; H8;
42 – 50	clay.

*The wetness of a peat is indicated by a scale of 5 degrees. W1 indicates a dry peat, W5 indicates a peat from which water may be easily squeezed by hand.

Humification of peat is indicated by a scale of 10 degrees. H1 is a raw unhumified peat, H10 is a completely humified peat without visible plant remains.

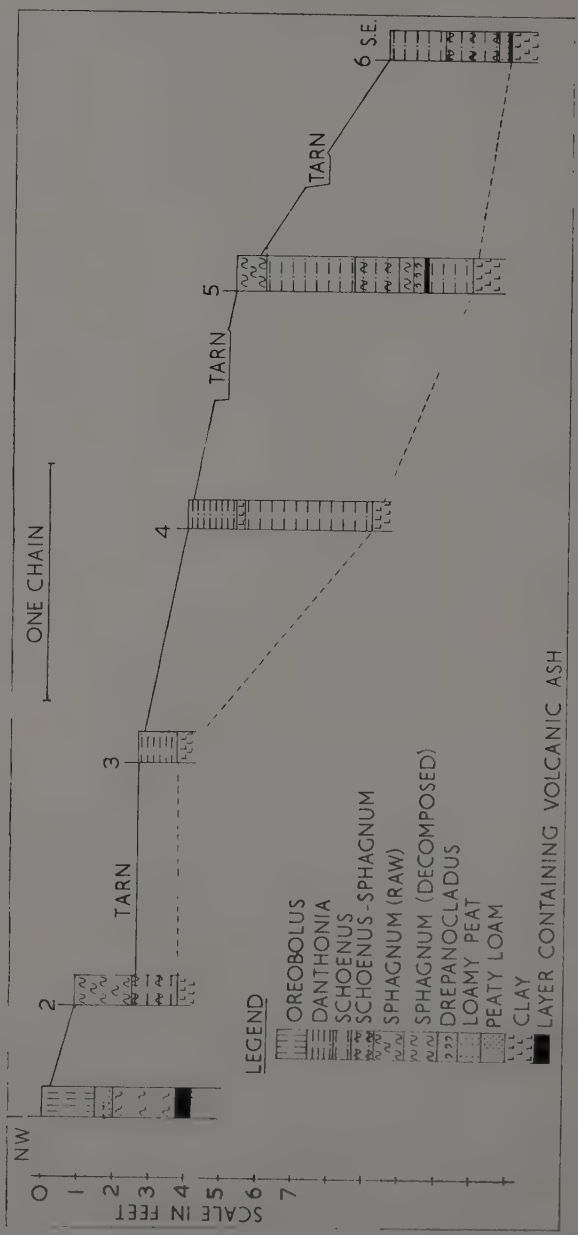

Fig. 2.--Peat profiles, mire 2, Mokai Patea ridge. The horizontal scale is in chains, the vertical scale in feet.

In the second profile, which was 22 in. deep, *Sphagnum* remains were not frequent. Three layers were distinguished in this profile; a shallow surface layer containing many fragments of *Carpha alpina* and *Oreobolus pectinatus,* a second layer in which bases of *Schoenus pauciflorus* were numerous, and a bottom layer containing fragments of *Carpha alpina* and *Oreobolus pectinatus,* resting on clay.

Mire 2

This mire, not far from the base of the steep slope leading to the summit of Rongotea (Fig. 1), extended into a steep narrow valley and was studied in some detail, six borings being made along a levelled line which crossed three tarns, but which was not continued down into the valley. The depth of peat varied considerably (Fig. 2), and the underlying stratum was blue clay in which were occasional small sharp-angled rock fragments. Recognizable fragments of *Schoenus pauciflorus* were plentiful; *Sphagnum* remains were generally not common except in borings made close to the tarns, although some *Sphagnum* remains were present at all depths. To illustrate such differences the results of a boring made close to the edge of a tarn, and those of a boring made some distance from a tarn are recorded.

Bore-hole 2, at edge of tarn.

Depth (inches)	
0 – 15	*Sphagnum* peat; *Schoenus pauciflorus* present; W5, H2;
15 – 20	*Sphagnum* peat; a few bases of *Schoenus pauciflorus;* W4, H6;
20 – 35	loamy peat; *Schoenus pauciflorus* fragments and some *Sphagnum* present; H4;
35 – 40	blue clay; this layer was not penetrated.

Bore-hole 4, between two tarns.

Depth (inches)	
0 – 16	fibrous, *Danthonia* and *Schoenus pauciflorus* fragments; W5, H7;
16 – 19	transition layer with small pumice particles present;
19 – 20	blue clay; some pumice;
20 – 60	slightly fibrous; some pumice; W2, H8;
60 – 62	transition layer;
62 – 70	blue clay.

In the profile at bore-hole 5 there was a 4-in. layer of *Drepanocladus* at depth of 5 ft. On the valley floor, and on one side of the valley, peat overlying greywacke contained many bases of *Schoenus pauciflorus,* the dark-red bases of this sedge giving the peat a characteristic colour.

Mire 3

Peat accumulation had ceased on this mire, and it was so densely overgrown by *Danthonia flavescens* that the possibility of there being peat in the area was overlooked until a sink-hole was discovered, the

sides of which were clearly peat. Layers of volcanic ash were visible in the profile, which contained much *Sphagnum*.

Details of the profile are recorded:

Depth (inches)	
0 – 6	peaty loam;
6 – 12	*Sphagnum* peat; at 11 in. a half-inch layer of fine, black, volcanic ash; H2;
12 – 38	fibrous, *Sphagnum* leaves, monocotyledonous remains, and small wood fragments; H6;
38 – 40	transition layer;
40 – 48	*Sphagnum* peat; *Schoenus pauciflorus* bases; wood fragments at 43 in.; yellowish volcanic material at 44 in.;
48 – 50	fibrous, laminated peat; wood fragments; H7;
50 – 64	fibrous, some *Schoenus pauciflorus* bases; H4;
64 – 68	clay; this layer was not penetrated.

Peat Formation

Peat formation has been a complex process in which continued saturation of the soil and the formation of tarns have been important factors.

The history of the smaller mires is closely linked to tarn formation. Rigg (1922) discussed the formation of ponds on a bench at a high altitude and concluded that they had been formed by the growth of ridges of vegetation composed of sedges and *Sphagnum*, which had impounded water seeping from higher slopes. Tarn formation on the Mokai Patea has followed a similar pattern. Water has accumulated in surface irregularities, and by the growth of *Sphagnum* in these wetter places a dam has been formed. Continued upward and lateral growth of *Sphagnum* has eventually resulted in the formation of a tarn, and water seeping through the dam has flowed down-slope to create peat-forming conditions and to maintain peat-forming vegetation.

On the larger mires, tarns appear to have been secondary, and not to have initiated peat formation, for there is no evidence of extensive *Sphagnum* accumulations in the deeper peat layers. Frequent rain, and water seeping from surrounding slopes, would maintain the gentle slopes, now underlying peat, in a constantly saturated condition so that peat formation could be initiated. On the slowly accumulating peat, tarns would be formed, first as small pools in surface depressions, but later, through the growth of *Sphagnum* in such situations, larger bodies of water would be impounded. Water draining from the tarns would then contribute to the maintenance of the peat-forming vegetation, and, consequently, to maintenance of the mire itself.

Discussion

The cycle of peat formation, accumulation, stagnation, erosion, and regeneration, has been emphasized recently by Osvald (1952) and Cranwell (1953). The present condition of the mires suggests that

peat formation and the extent of peat were greater at some time in the past, for peat formation is not now active and there is no widespread accumulation of peat. Stagnation and erosion are now the characteristic features of these peats.

It is evident that the two mires that are still accumulating peat depend mainly on the reserves of water in the tarns, for apart from these two there is no active peat formation in the locality. On mire 2 there are many muddy hollows under which *Sphagnum* remains are sometimes numerous, and, in others, there are scattered cushions of *Gaimardia pallida*. In the valley below, fibrous peat is being eroded on the valley side, and on the valley floor by a stream which now flows under peat and over a greywacke substratum. Hollows around the periphery of this bog were found, and the third area examined had long since ceased growth, and a deep hole leading to an underground drainage channel was evidence of erosion.

The vegetation itself suggested that optimum conditions for peat formation no longer exist. A small sloping bog near the crest of the main ridge was eroding at its upper surface, and dead *Sphagnum* mounds on the lower slope were being overgrown by *Dracophyllum recurvum* and by *Danthonia flavescens*. Mire 3 has already been discussed. The vegetation was so like the surrounding tussock vegetation that the presence of peat was at first not suspected. *Gaimardia pallida* occurred on the floor of existing tarns, and, where this species occurred on eroding peat, it suggested that the present surface was formerly the floor of a tarn.

The tarns also follow a cycle of formation and erosion. Death of the *Sphagnum* combined with the pressure of water in the tarn would result in the collapse of the dam, which would leave a large area of bare peat open to erosion or to colonization by bog plants. Trampling by deer would also lead to the erosion of an unstable system of tarns. There was no evidence that tarn formation was active, or that bare areas were being colonized.

Available data indicate that the mires have been formed under wetter conditions than obtain at present, and that the present state of erosion is likely to persist, although the two larger mires may maintain themselves as long as the tarns remain.

ACKNOWLEDGEMENTS

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SEED DISINFECTION

XII. GLASSHOUSE TESTS FOR CONTROL OF DAMPING-OFF OF *Pinus radiata* SEED

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(Received for publication, 30 September 1955)

Summary

In pathogenicity tests *Pythium* and *Pellicularia* caused damping-off of *Pinus radiata* seed.

Seed dressings did not increase total emergence under the conditions of experiments except in soil inoculated with *Pythium* and *Pellicularia*.

Seed treatments, although not completely inhibiting post-emergence infection, gave a significant reduction in the percentages of diseased seedlings.

The most successful treatments were those based on organic mercury, captan, and chloranil.

Introduction

Poor emergence of seedlings of *Pinus radiata* L., caused by damping-off fungi, is common in forest nurseries. Experiments here reported were carried out to assess the effect of seed disinfectants in protecting *P. radiata* seed from damping-off.

Materials and Methods

Laboratory tests carried out with 500 untreated seeds planted on filter paper and on malt-extract agar incubated at 20°c. gave 74% to 87% germination. Plating tests showed all seeds to be contaminated with *Penicillium* spp. and miscellaneous bacteria, whilst the incidence of other organisms was low, viz., *Mucor* spp. 0.4%, *Aspergillus* spp. 1.4%, *Rhizopus* spp. 0.2%, *Cladosporium* spp. 0.6%, and *Fusarium* spp. 0.6%. These organisms have no detrimental effect on emergence under normal sowing conditions.

The following seed dressings were used:

Captan: Orthocide 75 (75% captan);

Dithiocarbamates: Fernasan (50% thiram), Premasan (50% thiram), and Thirodust (70% thiram).

Organic mercury compounds: Agrosan (1% Hg as phenyl mercury acetate), Betanal (2.8% Hg as methoxy ethyl mercury silicate), Ceresan (1.7% Hg as phenyl mercury acetate and ethyl mercury chloride), Kleenseed (1.2% Hg as ethyl mercury chloride), and Leytosan (1% Hg as phenyl mercury chloride).

Quinones: Phygon XL (50% dichlone), Spergon (98% chloranil), and Tetroc (98% chloranil).

Materials were applied as percentages of seed weight in a rotary shaker. To secure adherence of the dressings, a preliminary treatment

was applied with a resin methylated sticker (Jacks, 1952) at 2% of seed weight. The percentage of seed dressings which adhered was assessed by re-weighing. Concentrations used are presented in the tables of results. Checks were set up with and without sticker.

Nursery soil based on light volcanic loam was used in the tests. Soil was prepared by the method given earlier (Jacks, 1951) and maintained at 35% water-holding capacity throughout the tests. Three series of tests were made: (1) in steam-disinfected soil (15 min. steaming at 200°F. ± 10°F.); (2) in untreated soil and (3) in steam-disinfected soil inoculated with *Pythium* and *Pellicularia,* as Roth and Riker (1942) found these organisms to be the principal causal agents of damping-off of pine seed. Soil was inoculated with *Pythium ultimum* Trow and *Pellicularia filamentosa* (Pat.) Rogers, (this organism was formerly known as *Rhizoctonia solani* Kuhn.) by mixing steam-disinfected soil and sand-oatmeal cultures. Emergence tests were carried out in tins, each holding 1 kg of soil. Five replications each of 40 seeds were used for each treatment. Glasshouse temperature throughout the tests was 65 ± 11°F. Five weeks after sowing, records were made of total emergence and of seedlings showing symptoms of post-emergence

TABLE 1. PHYTOTOXICITY TEST. EFFECT OF SEED DRESSINGS ON EMERGENCE OF *P. radiata* SEED IN STEAM-DISINFECTED SOIL

Treatment	Concentration %	Emergence		Treatment	Concentration %	Emergence	
		%	Equiv. angle			%	Equiv. angle.
Check + sticker	—	71.5	57.8	Check + sticker	2.0	86.0	67.9
Agrosan	17.0*	35.5	36.4	Orthocide 75	17.0*	90.4	72.0
,,	8.5	79.0	63.1	,,	8.5	90.8	72.6
,,	4.25	85.0	67.4	,,	4.25	84.4	66.8
,,	2.0	82.5	65.3	,,	2.0	86.0	68.1
Betanal	17.0*	0.0	0.0	Phygon XL	30.0*	61.5	52.0
,,	8.5	1.5	7.0	,,	15.0	67.5	55.5
,,	4.25	5.5	13.4	,,	7.5	63.7	56.4
,,	2.0	8.0	14.8	,,	2.0	71.5	57.8
Ceresan	17.0*	76.5	61.2	Premasan	20.0*	86.5	68.7
,,	8.5	80.0	63.6	,,	10.0	90.0	71.9
,,	4.25	76.5	61.5	,,	5.0	87.0	69.4
,,	2.0	78.5	62.9	,,	2.0	85.0	67.4
Fernasan	20.0*	73.5	59.1	Spergon	20.0*	80.0	63.5
,,	10.0	82.5	65.5	,,	10.0	84.0	66.6
,,	5.0	81.5	64.7	,,	5.0	86.0	68.2
,,	2.0	80.0	64.5	,,	2.0	74.0	59.7
Kleenseed	17.0*	82.0	65.9	Tetroc	20.0*	77.0	61.7
,,	8.5	85.5	67.1	,,	10.0	82.0	65.5
,,	4.25	79.0	63.5	,,	5.0	82.5	65.3
,,	2.0	81.0	65.3	,,	2.0	78.5	62.7
Leytosan	17.0*	83.0	66.0	Thirodust	20.0*	86.0	69.1
,,	8.5	76.5	61.4	,,	10.0	80.0	64.0
,,	4.25	85.0	67.5	,,	5.0	78.5	63.0
,,	2.0	79.0	63.9	,,	2.0	89.5	71.3
Sig. diff. at 1%		9.9					9.9

*Possible maximum amount adhering to seed.

damping-off. The latter included plants showing minor lesions as well as those which had caused death of seedlings.

RESULTS

Phytotoxicity Test

Betanal severely depressed emergence at all concentrations used (Table 1.). Agrosan at the highest concentration, and Phygon XL at all concentrations, also significantly depressed emergence in this soil. No treatment significantly increased emergence over checks, and no post-emergence infection occurred in this test.

Untreated-soil Test

This test was carried out in soil taken from a bulk lot prior to steaming. As facilities did not permit the use of all materials, this and further tests were carried out with one material representing each chemical group viz.; Ceresan (organic mercury), Fernasan (thiram), Orthocide 75 (captan), Phygon XL (dichlone), and Spergon (chloranil). Results are given in Table 2. Treatments neither depressed nor increased emergence. All treatments, however, reduced post-emergence infection as compared with checks. Isolations from diseased seedlings showed *Pythium* and *Pellicularia* as the main causal organisms.

TABLE 2. UNTREATED SOIL TEST. EFFECT OF SEED DRESSINGS ON EMERGENCE OF *P. radiata* SEED

Treatment	Concentration %	Emergence		Diseased seedlings	
		%	Equiv. angle	%	Equiv. angle
Check + sticker		75.0	60.2	58.4	49.9
Ceresan	17.0*	70.0	56.9	24.5	31.8
,,	8.5	72.5	58.4	21.5	26.9
,,	4.25	75.0	61.0	36.3	35.7
,,	2.0	77.5	61.9	20.0	26.1
Fernasan	20.0*	79.5	63.5	23.3	28.9
,,	10.0	74.0	59.5	22.2	28.1
,,	5.0	67.0	55.2	30.8	33.7
,,	2.0	78.5	63.0	29.6	33.0
Orthocide 75	17.0*	67.5	55.4	25.0	30.0
,,	8.5	72.5	58.4	23.5	29.0
,,	4.25	78.2	62.4	21.2	27.3
,,	2.0	80.0	63.5	28.6	32.4
Phygon XL	30.0*	63.5	53.2	30.7	33.4
,,	15.0	75.0	60.2	16.3	22.5
,,	7.5	72.5	58.9	30.8	33.6
,,	2.0	83.5	66.6	20.8	26.5
Spergon	20.0*	63.0	52.7	32.5	34.3
,,	10.0	65.5	54.4	27.4	29.8
,,	5.0	62.5	52.5	24.2	26.2
,,	2.0	72.9	59.1	25.8	30.4
Sig. diff. at 1%:			12.8		14.0

*Possible maximum amount adhering to seed.

Pathogenicity Test

Ceresan, Orthocide 75, and Spergon increased emergence in soil inoculated with *Pythium*, whilst Fernasan and Phygon XL were ineffective. In soil inoculated with *Pellicularia*, no increase in emergence resulted from seed treatment. Use of Phygon XL at higher concentration resulted in decreased emergence as compared with check. Ceresan, Orthocide 75, and Spergon consistently reduced the number of diseased seedlings for both pathogens, whilst Fernasan and Phygon gave inconsistent results (Table 3).

TABLE 3. PATHOGENICITY TEST. EFFECT OF SEED DRESSINGS ON EMERGENCE OF *P. radiata* SEED IN SOIL INOCULATED WITH FUNGI CAUSING DAMPING-OFF

Treatment	Concentration %	*Pythium*				*Pellicularia*			
		Emergence		Diseased seedlings		Emergence		Diseased seedlings	
		%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle
Check + sticker		63.0	52.7	37.5	37.6	77.5	61.2	11.5	19.1
Ceresan	17.0*	87.6	68.1	5.5	15.1	71.5	57.8	5.8	11.9
,,	8.5	85.0	70.5	8.7	15.9	76.5	61.0	4.7	12.1
,,	4.25	87.5	70.1	6.8	14.2	71.0	57.6	4.2	11.7
,,	2.0	83.5	68.8	13.2	20.9	87.0	71.3	5.7	13.3
Fernasan	20.0*	74.5	59.9	27.6	30.8	59.5	50.7	3.6	6.9
,,	10.0	56.0	48.6	15.6	18.0	69.0	56.5	7.0	15.1
,,	5.0	54.5	47.6	28.3	31.7	78.5	62.5	3.9	8.6
,,	2.0	65.0	53.8	15.8	19.7	86.5	68.9	10.1	17.0
Orthocide 75	17.0*	84.5	67.7	13.0	21.1	75.0	60.0	5.1	13.2
,,	8.5	86.5	68.3	11.2	19.2	77.5	62.0	4.0	11.5
,,	4.25	85.0	67.2	10.5	18.6	82.2	65.4	3.2	10.3
,,	2.0	87.5	68.5	14.0	21.2	87.5	69.1	2.6	9.3
Phygon XL	30.0*	46.0	42.7	40.3	39.2	44.0	41.5	7.4	15.6
,,	15.0	53.5	47.0	46.0	41.6	41.0	39.5	4.0	12.5
,,	7.5	45.0	41.7	45.5	42.5	59.5	50.2	6.7	14.4
,,	2.0	56.0	48.2	35.6	35.4	71.0	57.5	6.7	11.2
Spergon	20.0*	85.0	67.1	13.5	21.2	78.0	62.8	5.3	10.0
,,	10.0	88.0	70.5	10.4	18.3	74.5	59.9	2.9	8.7
,,	5.0	87.5	70.1	13.7	21.4	84.5	68.0	3.1	8.9
,,	2.0	84.5	67.2	14.6	21.2	77.0	61.5	3.8	9.8
Sig. diff. at 1%:			14.3		16.2		10.7		5.6

*Possible maximum amount adhering to seed.

Final Test

Ceresan, Orthocide 75, and, to a lesser extent, Spergon, increased emergence. Fernasan was ineffective, and Phygon reduced emergence. All treatments with the exception of Phygon XL in *Pythium* soil effectively reduced the amount of post-emergence damping-off (Table 4).

TABLE 4. FINAL TEST. EFFECT OF SEED DRESSINGS APPLIED AT 2% OF SEED WEIGHT ON EMERGENCE OF *P. radiata* SEED IN SOIL INOCULATED WITH DAMPING-OFF FUNGI

Treatment	*Pythium*				*Pellicularia*			
	Emergence		Diseased Seedlings		Emergence		Diseased Seedlings	
	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle	%	Equiv. angle
Check + sticker	78.0	62.7	49.8	44.6	72.0	59.0	53.4	46.8
Ceresan	83.6	67.0	16.3	23.5	84.0	66.6	22.1	26.2
Fernasan	76.8	61.4	17.8	23.5	67.6	56.6	28.5	31.0
Orthocide 75	84.0	66.5	21.4	26.5	80.2	64.0	29.6	31.7
Phygon XL	62.0	52.1	48.1	43.7	62.0	52.1	30.4	32.8
Spergon	78.4	62.7	23.8	28.6	84.8	67.4	15.4	22.4
Sig. diff at 1%:		4.0		8.1		4.8		8.9

CONCLUSION

Betanal proved phytocidal. Phygon reduced emergence in steamed but not in untreated soil. An increase in emergence brought about by treatments was recorded in inoculated soil. Treatments did not completely control post-emergence infection but the reduction obtained was substantial. Diseased seedlings recorded in treatments were also more vigorous and had smaller lesions than those from checks.

ACKNOWLEDGEMENTS

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SEED PRODUCTION STUDIES

I. THE EFFECT OF NITROGENOUS FERTILIZER, APPLIED AT DIFFERENT RATES AND DATES, ON SEED PRODUCTION IN COCKSFOOT (*Dactylis glomerata* L.)

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Summary

In cocksfoot (*Dactylis glomerata* L.), maximum panicle production followed autumn applications of nitrogen, but spring applications exerted a progressively greater influence in the following autumn as the trial proceeded.

Spring applications resulted in the greatest weight per 1000 seeds, and greatest number and weight of viable seeds per panicle. Date of application did not affect the number of seeds per unit area, or purity and germination percentages.

Increase in rate of application increased the number of panicles, the number and weight of viable seeds per panicle, and the number of seeds per unit area. Rate of application did not affect the weight per 1000 seeds or purity and germination percentages.

The relative weights of grain and pales were constant at widely differing weights per 1000 seeds.

The weight of viable seed per acre increased with increase in rate, and spring or split applications were more effective than autumn applications.

It is concluded that increasing rates of application are needed as the seed-production area ages, and that dividing the heavier applications between autumn and spring may be advisable.

Introduction

The average seed yield of cocksfoot (*Dactylis glomerata* L.) in New Zealand from 1946 to 1951 was 170 lb. per acre (N.Z. Census and Statistics 1952 a, b.), compared with average yields in Britain ranging from 390 to 600 lb. (Moore 1943; Watson and More 1944; Welsh Plant Breeding Station 1944). Andersen (1949) reported an average Danish yield of 490 lb.

Overseas workers have recorded increases in seed yield from applications of nitrogenous fertilizer, but their findings regarding the effect of date of application are conflicting. Schwanbom and Froier (1949) state that Hansen, in Denmark, obtained the largest increase in yield from the largest amount of nitrate of soda used, equivalent to 475 lb. per acre of sulphate of ammonia; he considered it better with large doses to give half in autumn and half in spring. They report also that Andersen, in contrast to Hansen, did not find any advantage in dividing the applications between autumn and spring, but recorded increasing yields from increasing doses of nitrate of soda up to the maximum dressing used, equivalent to 430 lb. sulphate of ammonia. Fulkerson *et al.* (1951), using five different nitrogenous fertilizers in spring, at the rate of 40 lb. nitrogen per acre, obtained increases of some 30% from all forms. MacVicar and Gibson (1951), with five forms of nitrogenous fertilizer at five rates (20 to 320 lb.N) in spring, found

that 80 lb. nitrogen gave optimum results, and no significant differences between forms were established. Evans (1953) showed responses in S.37 cocksfoot to all rates of application of sulphate of ammonia up to the maximum rate applied (8 cwt.), though in 1948 there was no additional response above 4 cwt., and in 1949 and 1950 little additional response above 6 cwt. Evans (1954) found that 4 cwt. per acre of sulphate of ammonia increased seed yield of S.37 cocksfoot by 2 to 4 cwt. per acre. A single spring application was better than an autumn application, while equal division between spring and autumn gave a yield equal to that of a single spring application.

In New Zealand, Sears (1950) reported that applications of 1 cwt. sulphate of ammonia in autumn and 2 cwt. in spring doubled the seed yield. On an area at the Grasslands Division Station, Gore, equal autumn and spring dressings of sulphate of ammonia were applied after the first harvest, at the rate of 1½ cwt. in 1949 and 2 cwt. in 1950. Seed yield averaged 400 lb. per acre over the three harvests and did not diminish over the period.

Most of the New Zealand harvest is from the C.23 and Akaroa strains of cocksfoot. Lambert and Thurston (1952) reported that the Grasslands Division strain (Corkill 1954) was intermediate in seed yield between these two strains. Unpublished data from the same trial showed that the Akaroa and the Welsh Plant Breeding Station strain, S.37, had similar seed yields.

The present experiment was designed to study the effect on the seed yield of cocksfoot of applying three rates of sulphate of ammonia or nitro-lime in autumn, spring, and equally divided autumn and spring dressings. The effect on some of the plant characters that determine weight of seed per acre was investigated.

Sulphate of ammonia and nitro-lime are equivalent in nitrogen content, and 1 cwt. of either supplies approximately 23 lb. nitrogen.

EXPERIMENTAL DESIGN AND PROCEDURE

The experimental area was at Gore on a recent alluvial sandy silt loam of the Mataura series (Cutler 1950), and, since it overlies gravel, soil moisture is deficient in a dry summer. This soil has shown marked response to initial phosphate applications, no response to potash, and little response to lime. The average annual rainfall recorded at Gore is 34 in. This is well distributed throughout the year but is heavier in summer than in winter.

The cocksfoot was sown at the end of February 1950, in rows 24 in. apart, at 3 lb. per acre, with 2 cwt. superphosphate. In early October 1950, a uniform dressing of 2 cwt. sulphate of ammonia per acre was applied. The pre-trial harvest in January 1951 yielded 415 lb. per acre of machine-dressed seed. Following this harvest the nitrogen treatments were started. Intercultivation kept the rows open, and reduced any effects of the crop litter described by Sears (1950). The plots were 4 rows wide and 104 ft. long. Treatments were as follows:

Treatments were replicated four times in randomized blocks. Autumn applications were made at the end of March, and spring applications in early September. In the first two years sulphate of

SULPHATE OF AMMONIA OR NITRO-LIME
(cwt. per acre)

Treatment	Autumn	Spring	Total
1	0	0	0
2	2	0	2
3	1	1	2
4	0	2	2
5	4	0	4
6	2	2	4
7	0	4	4
8	6	0	6
9	3	3	6
10	0	6	6

ammonia was used, but in the final year nitro-lime (NH_4NO_3 + $CaCO_3$) was applied, because of a temporary shortage of sulphate of ammonia. Uniform autumn applications of superphosphate at the rate of 2 cwt. per acre were made each year. At harvest, the two outside rows were discarded, together with 18 in. from each end of the remaining two rows.

Stratified random sampling was used in each of the two rows of the plots. In 1952, five samples, each two feet in length, were taken per plot row. In 1953 and 1954, the same sampling method was used, but eight samples of one foot in length were taken per plot row. For the last two harvests a caliper-like sampling tool was devised, which had parallel fingers twelve inches apart when opened. When these fingers were pushed through the row and the tool closed, the sample was readily cut with a sickle. Samples were stored in paper bags in a glasshouse. After drying, the panicles in each sample were counted, and threshed with a small 6-in. drum built for this purpose. The threshed material was then cleaned with a small "Clipper" seed cleaner. The purity and germination percentages, and weight per 1000 seeds, were determined by the Seed Testing Station, Department of Agriculture, Palmerston North. In 1952 and 1953, two samples of seed per plot were tested by the Seed Testing Station. These were obtained by dividing at random the samples from each plot into two groups, and bulking within the groups. Since no significant differences were found in purity and germination percentages all samples from each plot were bulked in 1954, and one purity and germination analysis obtained per plot. Data relating to seed have all been calculated on a basis of viable seed or 100% germination. The average percentage of viable seed in the machine-dressed samples was 82%. Hence the addition of 25% to the seed yields in the tables gives the approximate machine-dressed yields.

RESULTS

Maturity

No effect of time or rate of application on date of maturity of the crop could be discerned.

Lodging

There was a small amount of lodging in the heavier spring fertilized plots, in the first year, sufficient to cause harvesting difficulties. These plots also had more green herbage at harvest time, in sufficient amount to be undesirable when the crop was harvested by reaper and binder.

TABLE 1. NUMBER OF PANICLES PER FOOT OF ROW

Date	Rate (cwt. per acre)	1952	1953	1954	Mean
	0	61.8	37.7	43.5	47.7
Autumn	2	70.3	52.6	45.9	56.3
Split	2	71.4	49.6	46.3	55.8
Spring	2	62.5	46.8	48.4	53.5
Autumn	4	81.0	62.2	50.0	64.4
Split	4	75.9	64.4	63.8	68.0
Spring	4	69.6	56.7	55.7	60.7
Autumn	6	84.8	71.3	61.5	72.5
Split	6	71.1	64.4	65.5	67.0
Spring	6	60.9	55.1	65.6	60.5
S.E. individual means ±		3.2	3.8	4.6	2.2
Sig. diff. at 5% level		9.3	11.2	13.3	6.3
2 cwt. mean		69.0	49.7	46.9	55.2
4 cwt. mean		75.5	61.1	56.5	64.4
6 cwt. mean		72.2	63.6	64.2	66.7
Autumn mean		78.7	62.0	52.5	64.4
Split mean		72.8	59.5	58.5	63.6
Spring mean		65.2	52.9	56.6	58.2
S.E. Rate and Date means ±		1.8	2.2	2.6	1.2
Sig. diff. at 5% level		5.4	6.5	7.7	3.6
Linear trend: Rates		—	**	**	**
Linear trend: Dates		**	**	—	**

Results of tests for linear trend: — = not significant.
(*) = Significant at 10% level.
* = " " 5% "
** = " " 1% "

Purity and Germination of Seed

There were no significant effects of any treatment on either the percentage of pure seed in the machine-dressed samples, or on the percentage germination of pure seed.

Relative Weights of Grain and Pales

A commercial "seed" consists of the grain enclosed in the pales. To measure possible changes in the proportion in weight of these two parts, 500 seeds from each of several samples of varying weight per 1000 seeds were dissected into their component parts and weighed. The relative weight of grain and pales was almost constant from seeds of widely varying weight per 1000 seeds.

TABLE 2. NUMBER OF VIABLE SEEDS PER PANICLE

Date	Rate (cwt. per acre)	1952	1953	1954	Mean
	0	203	180	222	202
Autumn	2	240	182	203	208
Split	2	237	177	220	211
Spring	2	243	222	217	227
Autumn	4	214	183	209	202
Split	4	229	231	204	221
Spring	4	274	246	195	238
Autumn	6	251	190	206	216
Split	6	253	241	212	235
Spring	6	254	259	216	243
S.E. individual means ±		14.2	16.9	9.8	5.9
Sig. diff. at 5% level		41.4	49.2	—	17.3
2 cwt. mean		240	194	213	215
4 cwt. mean		239	220	203	220
6 cwt. mean		253	230	211	231
Autumn mean		235	185	206	209
Split mean		240	216	212	222
Spring mean		257	242	209	236
S.E. Rate and Date means ±		8.2	9.7	5.7	3.4
Sig. diff. at 5% level		—	28.4	—	10.0
Linear trend: Rates			*		**
Dates		*	**		**

Emergence of Panicles

In the first year it was obvious that panicles were emerging earlier in some plots than in others. *In situ* counts of emerged panicles were made on 16 November 1951, and analysis of variance of the data showed that there were highly significant trends towards earlier emergence following autumn applications, and from increase in rate of application. No differences due to treatment were observed in subsequent years.

Other Factors

Tables 1–4 record the effect of the treatments on the number of panicles produced per foot of row, the weight per 1000 seeds, the number of viable seeds per panicle, and the weight of viable seed per acre. Table 5 summarizes these tables, together with a summary of the weight of viable seed per panicle. Overall means only are presented for the weights per 1000 seeds in Table 4.

TABLE 3. WEIGHT (LB.) OF VIABLE SEED PER ACRE

Date	Rate (cwt. per acre)	1952	1953	1954	Mean
	0	466	291	379	379
Autumn	2	647	396	383	475
Split	2	629	377	422	476
Spring	2	633	465	451	514
Autumn	4	665	478	422	522
Split	4	673	636	538	616
Spring	4	760	613	493	622
Autumn	6	772	548	524	615
Split	6	693	675	594	654
Spring	6	618	629	597	615
S.E. individual means ±		42.7	57.6	43.7	25.0
Sig. diff. at 5% level		124.5	168.3	128	73.1
2 cwt. mean		636	413	419	488
4 cwt. mean		699	576	484	587
6 cwt. mean		694	617	572	628
Autumn mean		695	474	443	537
Split mean		665	563	518	582
Spring mean		670	569	514	584
S.E. Rate and Date means ±		24.6	33.3	25.0	14.5
Sig. diff. at 5% level		—	97.1	73.7	42.2
Linear trend: Rates		—	**	**	**
Dates		—	(*)	(*)	*

In the statistical examination of results, the linearity and curvilinearity of responses to increasing rate of application or delay in date of application were tested. In no cases were the curvilinear responses significant, and the results of the linear tests only have been recorded. Where linear tests are shown to be significant, therefore, the interpretation is that the maximum level of response to the treatment concerned had not been reached.

TABLE 4. MEAN WEIGHT OF 1000 SEEDS (MG.)

Rate or Date	1952	1953	1954	Mean
Control	775	922	825	841
2 cwt.	797	921	876	865
4 cwt.	813	899	885	866
6 cwt.	802	893	881	859
Autumn	786	871	862	839
Split	797	908	873	859
Spring	829	934	908	890
S.E. Rate and Date Means ±	9.8	11.3	13.7	8.3
Sig. diff. at 5% level	28.7	33.1	39.9	24.1
Linear trend: Rates	—	—	—	—
Dates	**	**	*	**

TABLE 5. SUMMARY OF OVERALL MEANS FOR THE THREE HARVESTS, 1952–54

Rate or Date	No. Panicles per Foot	Weight per 1000 Seeds (mg.)	Weight of Viable Seed per Panicle (mg.)	No. of Viable Seeds per Panicle	Weight of Viable Seed per Acre (lb.)
Control	48	841	170	202	379
2 cwt.	55	865	185	215	488
4 cwt.	64	866	190	220	587
6 cwt.	67	859	198	231	628
Autumn	64	839	174	209	537
Split	64	859	191	222	582
Spring	58	890	209	236	584

DISCUSSION

Applications of nitrogen affected the number of panicles produced, the weight per 1000 seeds, and the number of seeds per panicle. The product of these factors is the yield of seed per acre.

Number of Panicles per Foot of Row

Autumn applications produced the greatest number of panicles in the early part of the trial, but their effect declined as the trial proceeded. The effect of 2-cwt. applications declined generally over the period of the trial, and in the 1954 harvest no significant effect of 2 cwt. applied at any time was obtained. It is considered that 2 cwt. applied in successive years is not sufficient to maintain an increased yield of fertile tillers. Though 2 cwt. applied in spring had no significant effect in any year, the effect of 6 cwt. in spring changed from non-significant in 1952 to significant in 1953 and 1954. A similar, but less marked, effect was obtained from the 4-cwt. spring dressing. The 4-cwt. and 6-cwt. split dressings also became progressively more efficient relative to autumn applications. This increase in efficiency of spring and split applications at the heavier rates appears to be due to the continued effect of these dressings in the immediate post-harvest period. Shortly after harvest, which varied from 14 to 21 January, a darker green colour of the herbage and an increase in vegetative growth was observed each year in plots that had received 3 cwt. or more of the fertilizer in spring. After the first harvest, therefore, spring applications exerted an effect in *autumn*, before the autumn applications were made at the end of March. That the earlier emergence of panicles from autumn applications did not persist into the second and third years is considered to be further evidence that spring applications were exerting an increasing influence in the succeeding autumn.

Except in the split dressings, the limit of response had not been reached at 6 cwt. Evans (1953), with spring dressings, obtained the greatest number of fertile tillers from 6 cwt. sulphate of ammonia, and a smaller number from 8 cwt. MacVicar and Gibson (1951), using five different forms of nitrogenous fertilizer, at rates ranging from 20 to 320 lb. nitrogen, found no significant effect of form or rate. The results from a later trial of Evans (1954) showed that at the 4-cwt. rate there was a tendency for the split applications to increase relatively to the other times of application. In the present trial it appears that the spring portion of the split dressings, at the heavier rates, exerted an early post-harvest effect, and the autumn portion provided a later and additional effect. It is possible that an earlier date of application for the autumn part of a split dressing would be advisable.

Weight per 1000 Seeds

The weight per 1000 seeds was heavier where nitrogenous fertilizer was applied in spring, and rate of application did not affect it. MacVicar and Gibson (1951) found that 20 lb. nitrogen did not affect the weight per 1000 seeds, while 40 lb. increased the weight. No further increase was found when the rate was increased progressively to 320 lb. nitrogen. Davies and Edwards (1953) in a one-year trial, reported that time of application had little effect, and that, while 2 cwt. in spring did not give significant increases, increasing the rate to 4 cwt. gave significantly

heavier seeds. In the present trial a similar increase occurred in the first year, but the differences were not significant.

Germination

The conclusion that nitrogen treatments do not affect the germination of the seed agrees with that of Davies and Edwards (1953).

Number of Seeds per Panicle

The number of viable seeds produced per panicle was at a maximum following spring applications. Though no significant differences between date means were detected in 1952, there was a significant trend in favour of spring applications. Only in 1953 was there any significant effect of rate in any individual harvest, but the three-year average showed a significant trend towards increase in numbers with increase in rate. Where panicle numbers increased as a result of increase in rate of application, the number of seeds per panicle increased. But increase in panicle numbers due to autumn application was followed by a decrease in the number of viable seeds per panicle.

Weight of Seed per Panicle

The same effects were seen in the weight of viable seed produced per panicle, since this is the product of individual seed weight and number per panicle. The beneficial effect of nitrogen was greatest from the spring applications, and from increase in rate, though rate was less important than time of application.

Weight of Seed per Acre

The weight of seed per acre is obviously the product of the number of panicles and the weight of seed per panicle. Since increase in rate increased both these, a highly significant trend towards increase in weight of seed per acre occurred when rates were increased. Though spring applications produced fewer panicles initially, the heavier weight per panicle more than compensated for this, so that a greater weight of seed per acre resulted. All the 2-cwt. applications in the first year, and 2 cwt. in spring in the second year, produced significantly greater weights per acre than the control. The effect declined as the trial proceeded, and by the 1954 harvest no significant effect of 2 cwt. applied at any time, was detected. This is attributed to the failure of the lighter spring dressing to influence panicle production, and, in the case of the lighter autumn dressing, to a progressively smaller influence on panicle production as well as a lower weight of seed per panicle. MacVicar and Gibson (1951) found no significant increases in seed yield from 20 lb. nitrogen, and significant increases at 40 lb. and 80 lb. Further increase in rate did not raise yield. Evans (1953, 1954) found increases from up to 8 cwt., but little additional response above 6 cwt., and found that a spring dressing was more effective than an autumn dressing.

The number of panicles produced was higher from the split dressings than from the spring dressings, except at the 2-cwt. rate, but the spring dressings produced a greater weight of seed per panicle. The result was that seed yields from split and spring dressings did not differ, except at the 2-cwt. rate, where it appears that the spring dressing is the most efficient; at the 6-cwt. rate the difference in favour of the

split dressing was not significant. This does not confirm Evan's (1954) conclusion that dividing 4 cwt. equally between autumn and spring resulted in a lower seed yield than 4 cwt. in spring. Though the differences were not significant, there were indications in the present trial that the 4-cwt. split application was becoming more efficient as the trial progressed. The 6-cwt. split and spring dressings did not produce significantly greater weights per acre than the 4-cwt. dressings at these dates, but there were indications that they might be more beneficial after several harvests. The only advantage seen at present in dividing the higher rates between spring and autumn, is in the reduction of leaf in the crops. In some cases lodging might be a factor, and in this regard the split application did not cause lodging in the one year when lodging was experienced.

The no-nitrogen treatment has shown a high yield throughout the trial compared with the New Zealand average. The method of harvesting would reduce to a minimum any harvesting losses, resulting in higher yields from all treatments than would be obtained under normal harvesting methods. It is probable also that the intercultivation was beneficial in controlling weeds and incorporating crop debris in the soil.

Melville and Sears (1953) and Sears (1953) have shown the huge nitrogen requirements for high-producing pastures and forage crops. It is evident that high yields from cocksfoot seed crops are no less dependent on an adequate nitrogen supply.

CONCLUSIONS

Actual rates of application of nitrogenous fertilizer to cocksfoot seed crops cannot be specified, since differences in soil fertility, and in the age of the crop, must be taken into account. Where cocksfoot is sown for seed production under good fertility conditions, it is probable that nitrogen need not be applied at sowing time, or in the first preharvest spring. At lower levels of fertility, 2 cwt. of a nitrogenous fertilizer may be advisable, even at sowing time. In subsequent years increasing doses appear necessary, and after some four or five harvests at least 6 cwt. may be needed for continued high production. The question of dividing the application between spring and autumn cannot be decided, but there are indications that this may be desirable at the heavier rates. Further work on rates of application, the time of application within autumn and spring, and the proportion between these seasons, is necessary.

Where summer soil moisture is deficient, as at times in parts of Southland, the maximum response to nitrogen will be reached at a lower level than where soil moisture is plentiful. Continued production will deplete the reserves of other elements, so that their addition to the soil may become necessary.

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SEED PRODUCTION STUDIES

II. THE EFFECT OF GRAZING ON SEED PRODUCTION IN COCKSFOOT (*Dactylis glomerata* L.)

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(Received for publication, 8 September 1955)

Summary

In cocksfoot (*Dactylis glomerata* L.), grazing increased the number of panicles, but reduced the weight per 1000 seeds, the number and weight of viable seed per panicle, and the weight of viable seed per acre.

The effects of grazing were not modified by time of application of nitrogen (autumn or spring).

Spring applications of nitrogen increased the number and weight of viable seed per panicle, but weight per 1000 seeds was not affected significantly by time of application.

It is concluded that grazing of cocksfoot may reduce the seed yield from intercultivated rows, but a greater reduction may occur where crop litter accumulates.

Introduction

Reports on the effect of grazing cocksfoot (*Dactylis glomerata* L.) have been as conflicting as those on the effect of nitrogenous fertilizing. Wallace (1936) considered that judicious grazing might do some good to a cocksfoot stand. McPherson (1948) attributed much of the decline in Banks Peninsula production to uncontrolled grazing, but considered that light, controlled grazing might have a beneficial effect on both yield and quality of seed. Andersen (1949) stated that grazing at the end of summer, in Denmark, reduced the next year's seed crop, but that the application of nitrogenous fertilizer in autumn neutralized the injurious effect of the grazing. The harmful effect of grazing was considered to be in the removal of protective old leaves. Evans (1950), in England, recorded increases in both seed yield and number of fertile tillers, from an aged stand of cocksfoot grazed in mid to late-winter, while mid-spring grazing reduced the yield. He attributed the beneficial effect of the grazing to the removal of dead stubble which otherwise might depress production of shoots. Sears (1950) considered that accumulation of dead stubble prevented the replacement by seedlings of old plants as they thinned, and might allow invasion of twitch grass. Also, since the larva of the cocksfoot stem-borer moth (*Glyphipteryx achyloessa*) overwinters in stubble, recommendations for control involved the destruction of stubble. The results of his trial showed that both burning and clearing off the stubble in autumn opened up the sward, and allowed the establishment of tares in considerable quantity, especially where superphosphate was applied. Spring burning checked the tares, but all mechanical treatments reduced seed yield as compared with the undisturbed control. The addition of nitrogen greatly reduced the effect of the mechanical treatments. Johnson (pers. comm.), investigating causes of poor germination of cocksfoot seed, found that a thrips could cause considerable damage. She examined panicles from a current field trial in Southland, conducted by the author in collaboration with the Extension Division, Department of Agriculture, and her report

indicated that grazing in winter had resulted in a small decrease in thrips infestation, while burning the stubble in autumn caused a much greater reduction. Grazing also resulted in an increase in the number of panicles, but removal of the litter by autumn burning increased the number to a much greater extent.

Weaver and Rowland (1952), working on ungrazed native pasture, found that removal of plant debris resulted in a two-thirds increase in the stems of big bluestem (*Andropogon gerardi*) and switchgrass (*Panicum virgatum*). They quote Curtis and Partch as obtaining a sixfold increase in flower production, when old litter was removed from the crowns of switchgrass either by fire or clipping. Observation in Southland cocksfoot crops suggests that the accumulation of litter causes considerable reduction in the number of panicles. Lambert and Thurston (1952) reported a fall in seed yield after successive applications of nitrogen at high rates, in the absence of burning or grazing, and concluded that the considerable amount of straw and leaf litter had caused the fall in yield. Evans (1953), in studying the effects of close defoliation by cutting, found that the number of fertile tillers was increased with defoliation but the weight of seed per tiller was reduced. The time of cutting had an important effect, the greatest increase in fertile tiller numbers resulting from mid-September defoliation. Since nitrogen applications increased the weight of seed per tiller, he considered that nitrogen should be applied to a defoliated crop to maintain high levels of seed yield.

Experimental Design and Procedure

The area used was at Gore on the same soil type as described in Part I. The strain of cocksfoot and the method of sowing in rows were also the same, but the stand was an older one. Three harvests, with an average yield of 400 lb. dressed seed per acre, had been taken before the experiment started. After the first harvest, sulphate of ammonia had been applied in 2 cwt. dressings each autumn and spring up to 1951. The first experimental harvest in 1952 thus followed autumn plus spring applications of sulphate of ammonia. For 1953 and 1954 harvests the grazing plots were split so that respective halves received their nitrogen either in spring or in autumn. Nitro-chalk, equivalent to 4 cwt. per acre of sulphate of ammonia, was used in the last two years. A uniform dressing of 2 cwt. per acre of superphosphate was applied each autumn.

The plots were six rows wide, the centre pair of rows being used for measurement. They were 148 ft. long after 18 in. had been discarded from each end. The split plots were 72 ft. long.

There were three grazing treatments, with six replications, in two adjacent 3 × 3 Latin squares.

1. Control—no grazing.
2. Autumn graze, in early March.
3. Autumn graze, plus winter graze in mid-July.

Mature sheep were used for grazing, and were left on long enough to remove all green herbage. The dead stubble, about 6 in. high, protected the plants from close grazing. The average duration of grazing was twelve days in autumn and two days in winter. The average stocking rate was 35 sheep per acre for the autumn grazing, and 55 per acre for the winter grazing.

Stratified sampling was used; for the first harvest seven samples, each two feet in length, were taken from each of the two measurement rows. In the second and third harvests, with split plots, six samples, each of one foot were taken from each of the two measurement rows. Harvesting, threshing, and testing for germination and purity, were carried out as described in Part I. Data relating to seed have been reduced to a viable seed basis.

The minimum intercultivation needed to control any seedling growth between the rows was practiced. This interfered to the least extent with any differential growth resulting from the treatments.

RESULTS

Maturity

No effect of any treatment on time of maturity was observed.

Lodging

The amount of lodging was very small and no effects of treatments were observed.

Purity and Germination of Seed

No significant effects of treatments on purity and germination were detected, and data are not presented.

Interaction of Grazing and Nitrogen Treatments

There was no evidence of any significant interaction between the grazing and nitrogen treatments. Responses to grazing did not change as a result of adding the nitrogenous fertilizer in autumn or in spring. For this reason, individaul grazing x nitrogen means are not presented.

Tables 1–4 present the effects of the treatments on the number of panicles, the weight per 1000 seeds, the number of viable seeds per panicle, and the weight of viable seed per acre. The effect of nitrogen treatments is included only where significant differences occur. Table 5 summarizes Tables 1–4 and includes a summary of the weight of viable seed per panicle, together with a summary of the overall nitrogen effects.

TABLE 1. NUMBER OF PANICLES PER FOOT OF ROW

Grazing Treatments (N treatments ignored)	1952	1953	1954	Mean
No Grazing	50.7	48.1	60.2	53.1
Autumn	57.9	56.9	57.8	57.6
Autumn + Winter	61.6	63.3	57.7	60.9
Standard Error ±	2.3	1.9	2.0	1.4
Sig. diff. at 5% level	8.0	6.5	—	5.0
Linear trend with increased grazing	*	**	—	**

Results of tests for linear trend:
— not significant
(*) Sig. at 10% level.
* " " 5% "
** " " 1% "

TABLE 2. WEIGHT (MG.) PER 1000 SEEDS

Grazing Treatments (N treatments ignored)	1952	1953	1954	Mean
No Grazing	761	885	866	837
Autumn	740	859	863	820
Autumn + Winter	738	857	869	821
Standard Error ±	10.3	7.7	5.6	5.7
Sig. diff. at 5% level	—	26.7	—	—
Linear trend with increased grazing	—	*	—	(*)

TABLE 3. NUMBER OF VIABLE SEEDS PER PANICLE

A. EFFECT OF GRAZING TREATMENTS; NITROGEN TREATMENTS IGNORED

Grazing Treatments	1952	1953	1954	Mean
No grazing	251	355	252	286
Autumn	233	257	230	240
Autumn + Winter	194	247	215	218
Standard Error ±	7.8	11.3	7.7	5.1
Sig. diff. at 5% level	27.0	39.1	26.5	17.7
Linear trend with increased grazing	**	**	*	**

B. EFFECT OF NITROGEN TREATMENTS; GRAZING TREATMENTS IGNORED

Nitrogen Treatments	1953	1954	Mean
Autumn	273	221	247
Spring	293	244	271
Standard Error ±	7.4	6.0	5.1
Sig. diff. at 5% level	22.2	18.2	15.2

TABLE 4. WEIGHT (LB.) OF VIABLE SEED PER ACRE

A. EFFECT OF GRAZING TREATMENTS; NITROGEN TREATMENTS IGNORED

Grazing Treatments	1952	1953	1954	Mean
No grazing	456	711	628	598
Autumn	484	597	550	544
Autumn + Winter	429	635	517	527
Standard Error ±	18.4	30.6	28.0	18.3
Sig. diff. at 5% level	—	105.8	96.9	63.3
Linear trend with increased grazing	—	—	*	*

B. EFFECT OF NITROGEN TREATMENTS; GRAZING TREATMENTS IGNORED

Nitrogen Treatments	1953	1954	Mean
Autumn	653	525	589
Spring	642	605	624
Standard Error ±	27.8	35.7	20.4
Sig. diff. at 5% level	—	63.3	—

TABLE 5. SUMMARY OF MEANS

A. EFFECT OF GRAZING TREATMENTS; NITROGEN TREATMENTS IGNORED. MEANS OF THREE HARVESTS, 1952–54

Grazing Treatments	No. Panicles per Foot	Weight per 1000 Seeds mg.	Weight per Panicle mg.	No. per Panicle	Weight per Acre lb.
No grazing	53.1	837	241	286	598
Autumn	57.6	820	197	240	544
Autumn + Winter	60.9	821	180	218	527

B. EFFECT OF NITROGEN TREATMENTS; GRAZING TREATMENTS IGNORED. MEANS OF TWO HARVESTS, 1953–54

Nitrogen Treatments	No. Panicles per Foot	Weight per 1000 Seeds mg.	Weight per Panicle mg.	No. per Panicle	Weight per Acre lb.
Autumn	58.9	857	213	247	589
Spring	55.9	873	237	271	624

Discussion

The results of this trial show that grazing had a marked effect on seed production, and that applying nitrogenous fertilizer in autumn or spring did not modify the effect of grazing.

Number of Panicles

The greatest number of panicles was produced after the double grazing, though no significant differences were seen in the 1954 harvest. The double grazing produced more panicles than the single autumn grazing, and the latter more than no grazing. This is in agreement with the conclusions of Evans (1950, 1953). The nitrogen treatments did not modify the effects of grazing treatments, nor result in any significant effect on panicle numbers. In Part I it was shown that autumn nitrogen increased the number of panicles more than spring nitrogen, initially, but that spring nitrogen exerted a progressively greater effect in *autumn* in successive harvests. Nitrogenous fertilizer had been applied to the present trial area, in both autumn and spring, for the last two pre-trial harvests and the first experimental harvest. It is probable that the spring portion of these dressings was influencing panicle production in the succeeding autumn by the time the change to single autumn or spring dressings was made. Evans (1953) found that defoliation by cutting increased the number of panicles, and other work in Southland (Johnson, pers. comm.) indicates that removal of litter by burning increases the number of panicles more than does grazing. It is concluded that the effect of grazing is from defoliation and litter removal rather than from any fertilizing effect of stock dung or urine.

Weight per 1000 Seeds

The weight per 1000 seeds declined with grazing. Differences were not significant in the 1952 and 1954 harvests, but attained significance in 1953 and in the three-year mean. In the latter two sets of data, a significant trend towards lowering of weight with increase in the number of grazings occurred. This effect appeared to be inversely related to the number of panicles, weight per 1000 seeds falling as the number of panicles increased. Application of nitrogen at different times did not modify the effect of grazing. Spring applications of nitrogen gave a higher mean weight than autumn application, as in Part I, but the difference was not significant.

Germination

Neither the grazing nor the nitrogen treatments affected germination of the seed.

Number of Viable Seeds per Panicle

The number of viable seeds per panicle decreased significantly with increasing severity of grazing. The effect of spring nitrogen was to increase markedly the number of seeds as compared with the autumn nitrogen. This agrees with the results in Part I. There was no interaction of grazing and time of nitrogen application, so that, while the effect of spring nitrogen compensated to some extent for the smaller number produced by grazing, a parallel increase was obtained for spring nitrogen on ungrazed plots, where the numbers were already higher.

Weight of Viable Seed per Panicle

The weight per panicle also decreased significantly with autumn grazing, and further with double grazing, a result which was not modified by the time of nitrogen application.

Under all grazing treatments the weight per panicle was significantly higher after spring nitrogen than after autumn nitrogen. Since time of application did not affect greatly the weight per 1000 seeds, the higher weight per panicle is due almost solely to increase in numbers of seeds from spring nitrogen. In Part I of this series, spring nitrogen also increased the weight of viable seed per panicle, but there the increase was due to significant increases in both numbers per panicle and weight per 1000 seeds.

Yield of Seed per Acre

The yield of seed per acre was significantly highest from the no-grazing treatment in the 1953 and 1954 harvests, and in the mean of the three years. A significant trend was seen from the 1954 harvest, and in the three-year mean, towards reduction in yield with increase in grazing. The most important factor in this was the marked reduction in the number of seeds per panicle and in the less-marked reduction in weight per 1000 seeds. The increase in panicle numbers following grazing was not sufficient to make up for these reductions. This confirms the conclusion of Evans (1953). In the two years when divided nitrogen applications were made, no interaction of grazing and nitrogen treatments occurred. In the 1953 harvest, time of application of nitrogen had no significant effect but in 1954 the spring nitrogen produced a significantly greater yield of seed. A study of the 1954 data shows that this increased yield came from the increase in weight per 1000 seeds, which affected weight per panicle. It is probable that the spring nitrogen would show the same increase in later years, a conclusion which agrees with that of Evans (1953). In the present trial the same increase was obtained with spring nitrogen application on the already higher-yielding no-grazing plots. A further nitrogen application to the grazed plots probably would be necessary to obtain as high a yield as from the ungrazed plots. The cost of a possible 2 cwt. extra of nitrogenous fertilizer might be offset by the value of the grazing obtained. The average grazing from the autumn period was 770 sheep days.

The present trial was conducted on intercultivated rows so that a build up of litter between the rows was prevented in any case. Moreover, the straw from the bulk of the crop, after sampling was finished, was removed from the area. In the normal commercial crop, sown in 7-in. drills, debris builds up into a litter, particularly under the modern tendency towards windrowing and later threshing with a pick-up attachment on a combine harvester. It is suggested that some means of eliminating this litter is necessary. Burning appears to be very efficient both as a litter remover and a destroyer of pests, but cannot be used indiscriminately. Preliminary trials with grazing on such a crop are encouraging, but the effect of the intensity and duration of grazing needs study.

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THE USE OF DRIED BUTTERMILK AS A FEED FOR DAIRY CALVES

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Summary

A 2 × 2 factorial type of experimental design was used to study the usefulness, as a feed for young dairy calves, of reconstituted buttermilk prepared from two types of dried buttermilk—one with a high fat content (16%) the other with a low fat content (8%)— when each reconstituted milk was fed at the rate of 10% and 12½% of body weight. The calves were fed whole milk for the first 4 weeks at the end of which time the whole milk was gradually replaced by reconstituted buttermilk. From 6 to 10 weeks of age they received only buttermilk. Throughout the whole period of the trial the calves grazed good-quality pasture. It was found that there was no significant difference between growth rates of the calves fed on the two types of powder. However, the feeding of the reconstituted milk at the rate of 12½% of body weight resulted in significantly better weight gains than feeding it at the rate of 10% of body weight.

Introduction

The drying of buttermilk has been widely practised by New Zealand dairy companies in recent years. Supplies in the domestic and overseas markets exceed the demand for this dairy by-product for incorporation in human foods, and this protein-rich food of milk origin is available for stock feeding. Many New Zealand farmers use it for feeding to calves with excellent results. It is often used by farmers producing milk for city supply, particularly during the winter months when liquid whole milk commands such a high price that, if fed to calves, the cost of raising home-bred replacement stock becomes too high. It is also used for feeding calves by farmers who sell milk to dried-milk plants. It could also fill a similar role in providing farmers who deliver milk to cheese factories with a substitute for whole milk or possibly as a supplement to whey. Thus there is need for investigating how dried buttermilk can be most profitably used as a calf food. In addition there is need for determining whether the fat content of dried buttermilk affects its food value, since this varies considerably and tends to increase towards the end of the dairying season. High fat content makes dried buttermilk unsuitable for incorporation in certain human foods because of the liability of the excess fat to oxidize.

The trial reported in this paper was designed to show whether dried buttermilk of a high fat content differed from one with a low fat content, as a food for young dairy calves.

Review of Literature

Buttermilk in New Zealand is obtained from the sweet or neutralized cream, pasteurized in flash-pasteurizers or vacreators, cooled quickly, and held overnight before churning. In the United States, much of the buttermilk is concentrated (as distinct from dried) either as condensed or semi-solid buttermilk. The latter product is used mainly for animal feeding. The buttermilk is ripened with acid-producing

starters for some time before it is concentrated, in order to build up the acidity. This high acidity serves to "pickle" the semi-solid buttermilk, i.e. to prevent deterioration (McDowall 1953).

Liquid buttermilk has been fed to calves for long periods. Archibald *et al.* (1916) found it slightly superior to skim milk, while Shepherd and Converse (1939) noted that although buttermilk has practically the same composition as skim it contained only a small amount of fat and a certain acidity.

Eckles and Gullickson (1924) conducted an experiment using a very small number of calves (five) of different breeds and sexes. They reared the calves on condensed or powdered buttermilk, each being diluted so as to give a total solids content similar to fresh buttermilk and each group was given hay and a grain mixture. Whole milk was fed for the first two weeks of life, the change over to the new ration being made gradually. All the calves made excellent weight gains and little trouble was experienced with scours or indigestion.

Ellington and Knott (1926) raised eight calves on a mixture of 3 lb. solid buttermilk to 9 lb. of water, and on a mixture of 1 lb. of dried buttermilk to 9 lb. of water, together with alfalfa hay and grain. The two buttermilks were found to be satisfactory substitutes for skim milk when this was not available.

Buttermilk powder has also been fed dry; Lindsey and Archibald (1929) found that in young calves it produced about the same rate of gain as skim-milk powder. It was more economical, but in some cases had a decided laxative effect.

In an experiment at Idaho (Anon. 1931), a group of Holstein calves was fed whole milk for two weeks and then gradually changed over to dried-buttermilk solution. Growth rates of the calves were similar to a control group receiving skim milk.

Woodman (1923) fed groups of 4 calves skim milk at the rate of one-fifth, one-sixth, and one-seventh of body weight and compared their growth rates with another group fed all the skim milk they would drink. The latter group made faster weight gains. It was noted that at the high level of feeding no undue scouring occurred.

McCandlish (1939) reviewed other trials, which showed that the more liberal allowances gave better results.

At the Dairy Research Institute (1946) very little difference was found between calves fed skim milk at either 10% or 15% of body weight, though in that year the season was favourable for calf rearing.

Thus, favourable results are reported in the literature from feeding buttermilk powder whether dry or reconstituted. However, many of the experiments reported have had too few calves per group and lack precise experimental techniques.

Experimental Methods

Animals Used. Thirty-two new-born calves of both sexes, mainly grade Jersey, were collected from the Manawatu area in January 1951. On arrival at the Institute 2-3 days after birth, the calves were earmarked

and assigned to the 4 groups as they arrived in a predetermined random order.

Experimental Design. The experiment was of a 2 x 2 factorial design in which two types of buttermilk powder after reconstituting with water were fed at two levels, 10% and 12½% of body weight. There were eight calves in each group.

Composition of the Powders. Table 1 shows the chemical analysis of the two types of buttermilk.

TABLE 1. COMPOSITION OF THE TWO TYPES OF BUTTERMILK POWDER

Constituent	Type A High Fat %	Type B. Low Fat %
Fat %	16.10	8.19
Total Protein %	28.54	32.69
Lactose %	39.62	44.58

Management. The powders were made by the spray-drying process at Awahuri Dairy Factory. The high-fat powder was freshly made while the low-fat powder had been prepared for some months. Both batches were packed in hessian bags with paper liners and an inner liner of plastic. The powders were not caked but were free running and had a clean fresh odour, there being no suggestion of oxidized-fat flavours.

Up till one month of age the calves were fed whole milk in two groups at the two rates of feeding. As they increased in age from four to six weeks, the whole milk was gradually replaced by dried buttermilk, reconstituted at the rate of 1 lb. of powder to 9 lb. of water. From six weeks till the experiment concluded at ten weeks of age, the calves received reconstituted buttermilk powder alone.

At all times the calves had access to good-quality pasture, care being taken to move them to fresh pasture every day. Body weights were recorded once a week on a scale accurate to 1 lb. Notes were kept on the health and appearance of the calves.

RESULTS

Fig. 1 shows the growth curves of the groups of calves. The points on the graph are the means of eight calves per group.

Since it was probable that larger calves with their larger appetites made faster weight gains, adjustment was made by means of covariance as outlined by Snedecor (1948), to overcome this source of bias in the data.

A preliminary analysis was made to determine whether birth weight or 4-weeks weight was the better to use as the independent variable when associated with the dependent variable, gain in weight 4 to 10 weeks. The correlation between birth weight and gain in weight 4 to 10 weeks was 0.39 which was significant at the 5% level. The use of 4-weeks

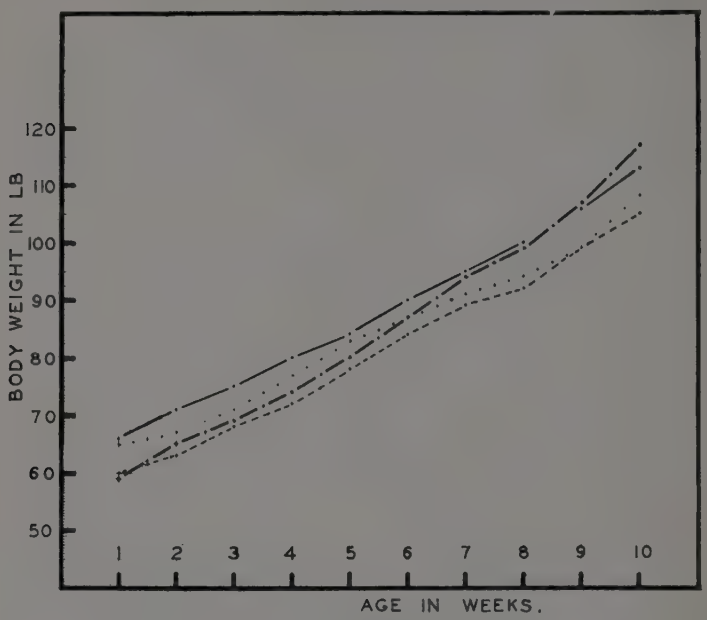

Fig. 1.—Average growth curve for each of the four groups of calves. —— . —— . high-fat buttermilk, 10% level of feeding. – – – – normal-fat buttermilk, 10% level. — . — . high-fat buttermilk, 12½% level. . . . . normal-fat buttermilk, 12½% level.

weight as the independent variable resulted in a correlation of 0.57. Both these correlations were derived on a within-treatment basis. Since all the calves received whole milk till four weeks of age, and the higher correlation resulting when 4-weeks weight instead of birth weight was used as the independent variable, a covariance analysis was made on the growth data using 4-weeks weight and gain in weight 4 to 10 weeks.

Table 2 shows the analysis of covariance with the 3 degrees of freedom for treatments, subdivided into one degree of freedom for levels, one for types of buttermilk powder, and one for the interaction of level × type.

An analysis of the error variance showed that the reduction in the error variance, due to the regression of gain in weight from 4 to 10 weeks associated with the weight at four weeks, was highly significant. The general mean for the gain in weight was 34.38 lb., the standard deviation derived from the within-treatment variance adjusted for regression was 7.97 while the coefficient of variation was 21.91%.

Thus the experiment showed that a significant difference in weight gains due to the two levels of feeding occurred, but no significant difference could be found between the weight gains on the two types of buttermilk powder. The following table shows the average gains in weight from 4 to 10 weeks adjusted for the regression.

TABLE 2. ANALYSIS OF COVARIANCE OF WEIGHT GAINS USING 4-WEEKS WEIGHT AND GAIN IN WEIGHT 4 TO 10 WEEKS

Source of Variation	d.f.	Sx	Sxy	Sy	Errors of Estimate s.s.	d.f.	m.s.	F.
Total	30	4809.97	1773.63	3075.50				
Treatments	3	298.77	—132.25	617.00				
Levels	1	3.78	— 38.49	392.00				
Buttermilk	1	47.53	72.13	112.50				
Levels × buttermilk	1	247.46	— 21.63	112.50				
Within treatments (error)	27	4511.20	1905.88	2458.50	1653.31	26	63.59	
Levels + error	28	4514.98	1867.39	2850.50	2078.15	27		
Levels					424.84	1	424.84	6.68*
Buttermilk + error	28	4558.73	1987.01	2571.00	1712.75	27		
Buttermilk					59.44	1	59.44	N.S.
Interaction + error	28	4758.66	1884.25	2571.00	1824.91	27		
Interaction					171.60	1	171.60	N.S.

*Significant at 5% level.

Note: One of the calves in the group on low-fat buttermilk died at the age of 8 weeks, which caused a loss of 1 degree of freedom in the analysis.

TABLE 3. ADJUSTED MEAN WEIGHT GAINS (LB.) 4 TO 10 WEEKS

Type of Buttermilk	Levels		Means for Buttermilk
	10%	12½%	
High-fat buttermilk	31.04	44.43	37.74
Low-fat buttermilk	34.42	35.60	35.01
Means for levels	32.73	40.02	

The difference in adjusted weight gains of the calves between the two types of buttermilk was 2.73 lb. in 6 weeks or 0.46 lb. per week. This difference was not significant. For levels, the difference was 7.29 lb. in 6 weeks or 1.22 lb. per week, and this difference was significant.

Between the ages of 2 and 5 weeks, nutritional scours affected some calves. However, there was no significant difference between the groups in the incidence of scours.

Discussion

From the graph of the average growth rates of each group of calves (Fig. 1) it will be seen that at 8 weeks of age the calves in two of the groups had reached a little over 90 lb. in weight. Part of the difference in average weight at 8 weeks would be due to some groups being heavier than others at the start of the experiment. The weight of these calves at 8 weeks, however, falls below the level regarded by McMeekan (1954) as optimum.

However, the evidence for his high standards of rearing is based on one experiment comparing set stocking versus rotational grazing up to two years of age, where there was an advantage in butterfat production in favour of the rotationally grazed animals. But set stocking for two years is quite different from rearing on a standard a little below that set by McMeekan for a period and then feeding them well at a later date. Because no measurements of intake were made, there is no information as to the relative efficiencies of the two types of rearing or of the lactating animals.

A period on a plane of nutrition which does not permit maximum rate of body development later giving way to a diet which permits growth to adult size has been shown by McCay (1942) with rats and Eckles and Swett (1918) with cows, very much to prolong life. Steensburg (1947) with heifers showed a compensatory accelerated growth rate after retardation for a period. Work with monozygous cattle twins with intake measurements (Bonnier, Hansson, and Skjervold 1948, and Hanson, Brännäng, and Claesson 1953) has shown that the efficiency of feed utilization decreases considerably with an increased level of nutrition, and that a low level of nutrition during rearing was adequate. Troubles with restricted life span (Brody 1945), and upsets to the reproductive system (Asdell 1949), have been experienced with overfeeding.

From the available evidence, there does not appear to be any optimum growth rate apart from one that will maintain health and thrift in calves. It seems that, provided the rate of growth is sufficient to maintain life and health over the first few months of a calf's life, subsequent nutrition in the form of an adequate supply of good-quality pasture would soon allow calves to gain in weight.

The calves in the trial reported in this paper were reared during late summer and early autumn, when the quality of the pasture would not have been as good as pasture supplied to calves born in spring. This may have caused somewhat slower growth rates, but there was no doubt that the calves were healthy and thrifty.

Feeding the reconstituted powder at the rate of 12½% of body weight resulted in significantly better weight gains than at the 10% level. It would have been desirable to try a high level of feeding, say 15% of body weight, but the number of calves did not permit this. The two lower levels were chosen as there was a chance of the 15% level causing scouring troubles, particularly as one of the powders used was of a high fat content.

As there was no significant difference between the gains on the low-fat buttermilk powder and on the high-fat powder, it appeared that the fat content of the powder did not adversely affect the welfare of the calves.

ACKNOWLEDGEMENTS

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FIELD TRIALS ON CONTROL OF HALO BLIGHT OF BEANS (*Pseudomonas phaseolicola* (Burkholder) Dowson)

By D. W. Dye, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 14 October 1955)

Summary

In field trials carried out to test the efficacy of therapeutants in controlling halo blight of beans on heavily infected bean plants, Bordeaux mixture 6-8-100, and streptomycin sulphate 75 p.p.m., controlled the disease in two successive seasons, Bordeaux giving the higher degree of control. Phygon XL 1 lb. per 100 gal. was not effective.

Both Bordeaux mixture and streptomycin sulphate significantly increased yield of pods in 1953-54 but in the 1954-55 season only the latter material gave a significantly higher yield than the control.

Introduction

The usual recommendations for the control of halo blight (*Pseudomonas phaseolicola* (Burkholder) Dowson) are the sowing of disease-free seed or the use of resistant varieties (Reid 1945; Elliot 1951). However, infection cannot always be detected in seed, and some of the highly susceptible varieties are the most popular with market gardeners. Reid and Taylor (1945) showed that four applications of Bordeaux mixture 6-8-100 reduced halo blight to a negligible amount when spray treatments were commenced before plants were infected. In glasshouse experiments Mitchell *et al.* (1952) and in field experiments Zaumeyer *et al.* (1953) showed that streptomycin sulphate prevented the development of halo blight.

In New Zealand, halo blight is spasmodic in its occurrence and protective sprays are not generally applied. It would therefore be advantageous if, in the event of heavy infection, therapeutant applications could control the spread of this disease. Trials to test this possibility have been carried out during two seasons.

Materials and Methods

On 4 November 1953 at Auckland, 16 plots (each of three rows 10 ft. long) were sown with Masterpiece dwarf-bean seed. When plants were at the first true leaf stage an inoculum of *Ps. phaseolicola* in Difco nutrient broth was sprayed over all plants. This was repeated six days later. By 9 December, 91% of plants were infected and 30% of the whole were classed as heavily infected with halo blight. Three therapeutant applications were then made at fourteen-day intervals. Therapeutants tested were:

1. Bordeaux mixture: 6 lb. copper sulphate, 8 lb. hydrated lime, 100 gal. water.
2. Streptomycin sulphate (720 units per mg. 75 p.p.m. streptomycin base.
3. Phygon XL (50% 2,3 -dichloro -1,4 - naphthoquinone) 1 lb per 100 gal.
4. Control—water only.

Treatments consisted of four replications randomized in blocks. Pods were harvested three times at seven-day intervals; pods from each row were counted, weighed, and examined for presence of halo blight. Any pod showing infection, irrespective of number of lesions, was recorded as "infected", no attempt being made to record severity.

In 1954–55 the experiment was repeated. Phygon XL was not included since the result in 1953–54 was unsatisfactory. Three inoculation sprays were applied; before the first therapeutant spray was applied, 85% of plants were heavily infected with halo blight. Five therapeutant applications were made at seven-day intervals. Harvesting was carried out as in the previous year.

RESULTS

The percentages of infected pods in each treatment are shown in Table 1. Bordeaux mixture and streptomycin sulphate efficiently controlled halo blight in both seasons, but Bordeaux mixture gave a higher degree of control than did streptomycin. Phygon XL was ineffective.

Crop yields are presented in Table 2.

In the first season, Bordeaux mixture and streptomycin treatments resulted in a highly significant increase in yield of marketable pods, but these treatments were not significantly different from each other. In the second season streptomycin-treated plots gave a significantly higher yield than Bordeaux mixture or control plots.

Streptomycin sulphate did not cause any plant damage in either season. Bordeaux mixture caused a hardening of the foliage and some marginal necrosis in both seasons, but the effect of plant damage on crop yields could not be assessed.

TABLE 1. THE EFFECT OF THERAPEUTANTS IN CONTROLLING HALO BLIGHT OF BEANS ON PODS (Percentage of infected pods)

	1953/54				1954/55			
	5/1/54	13/1/54	19/1/54	Mean	29/12/54	5/1/55	12/1/55	Mean
Bordeaux (6:8:100)	3.8	1.6	0.6	2.2	2.1	2.8	1.7	2.2
Streptomycin 75 p.p.m.	17.0	9.2	7.7	12.0	8.9	9.8	6.6	8.6
Phygon XL 1 lb./100 gal.	19.1	15.0	30.6	22.2				
Control	20.6	17.7	24.1	21.1	28.7	24.1	14.5	24.1
Differences for significance								
5%	6.5	6.5	6.5	3.8	6.1	6.1	6.1	3.5
1%	8.8	8.8	8.8	5.1	8.4	8.4	8.4	4.8

TABLE 2. THE EFFECT OF THERAPEUTANTS ON THE YIELD OF BEANS INFECTED WITH HALO BLIGHT (lb. per plot)

	1953/54				1954/55			
	5/1/54	13/1/54	19/1/54	Total	29/12/54	5/1/55	12/1/55	Total
Bordeaux (6:8:100)	8.3	4.7	6.4	19.4	11.8	7.6	4.8	24.3
Streptomycin 75 p.p.m.	6.9	5.1	5.3	17.4	14.5	9.2	6.2	29.9
Phygon XL 1 lb./100 gal.	1.1	2.3	2.9	6.3				
Control	1.4	2.3	3.1	6.7	11.8	7.8	5.1	24.7
Differences for significance								
5%	1.5	1.5	1.5	2.6	2.1	2.1	2.1	3.7
1%	2.0	2.0	2.0	3.4	2.9	2.9	2.9	5.1

DISCUSSION

It is apparent that, under Auckland conditions, halo blight of beans can be controlled after heavy infection has appeared on plants. Although Bordeaux mixture 6: 8: 100 gave a greater degree of control than streptomycin at 75 p.p.m. it caused damage to the plants. Concentrations of streptomycin considerably higher than 75 p.p.m. do not damage bean plants (M. H. Dye, pers. comm.) and it is likely that higher concentrations would control halo blight equally as well as did Bordeaux mixture 6-8-100. Three sprays of either Bordeaux mixture or streptomycin at fourteen-day intervals proved as efficient as five sprays at seven-day intervals in weather conditions prevailing during these trials.

Halo blight causes a considerable reduction in yield of beans if weather conditions are favourable for the development and spread of the causal organism, and under these conditions Bordeaux spray gives a significant increase in yield (Reid and Taylor 1945). The weather in 1954 55 was not favourable for the development of the disease, and consequently there was no significant difference between the Bordeaux plots and the check plots.

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A PRELIMINARY ACCOUNT OF *Dasyneura mali* Kieffer (Cecidomyidae : Dipt.), AND AN ASSOCIATED HYMENOPTEROUS PARASITE IN NEW ZEALAND

By D. H. Todd, Entomological Research Station, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 17 October 1955)

Summary

Parasitism of the apple leaf-curling midge, *Dasyneura mali* Kieffer, by a hymenopterous egg parasite, *Prosactogaster demades* Walker* is recorded for the first time. The parasite was found to be giving some degree of control during the 1954-55 season at Palmerston North, but further investigations will be necessary before full assessment of its potentialities can be made. Midge populations in New Zealand are higher than those recorded in the United States of America, where the insect is considered to be a pest of some importance.

Introduction

The apple leaf-curling midge *Dasyneura mali* Kieffer, which spread from Europe to North America and the Argentine, is now an established pest of apple trees in New Zealand. A hymenopterous parasite, *Prosactogaster demades* Walker, has been found to be attacking the apple leaf-curling midge in this country.

Morrison (1953) reports that in January 1950 a shipment of East Malling IX apple stocks of Dutch origin was planted out in a number of North Island nurseries. Two months later, the stocks planted in an Auckland nursery were found to be infested with apple leaf-curling midge. This insect has since appeared in many other orchard areas in the North Island.

The following account details observations made on *D. mali* and its parasite *P. demades* during the 1954-55 season. The investigation were made in an apple orchard situated at Massey College, Palmerston North.

Dasyneura mali Kieffer

Description and Damage

The adult midge is a typical medium-sized *Dasyneura* species, the female being 1.5 to 2.5 mm. long with iridescent wings 1.5 to 2.0 mm. in length. The abdomen is red with black scales dorsally, the legs and thorax brownish. The male is slightly smaller and lacks the reddish colour on the abdomen. The cigar-shaped egg is orange in colour. The larva is at first white to creamy white, the colour deepening to orange when mature. The pupa, which is enclosed in a tough silken cocoon, is also orange.

The midge is a specific pest of apple trees, and damage results from the rolling of the leaves, which then cease to function normally and may drop prematurely. Terminal leaves and watersprouts are most susceptible

* This parasite was previously known as *Misocyclops marchali*, but Mr. G. E. J. Nixon of the Commonwealth Institute of Entomology, who kindly identified the material, states that the name used in this paper has a prior claim.

to attack, older leaves and trees with little or no growth being seldom infested. Although severe stunting of apple stocks occurred in a number of Palmerston North nurseries during the current season, no noticeable reduction in the abundance of fruit was observed on infested bearing trees.

Life History

During the period when apple trees are producing a maximum amount of growth, the female midges lay their eggs in groups on the margins or upper surfaces of the uncurled or partly uncurled leaves. At the latter end of the season when practically all the new growth of the trees is confined to a limited number of watersprouts, oviposition sites are restricted and eggs are not deposited on any specific part of the leaf. Whitcombe (1934), states that each female is capable of laying up to 200 eggs, and the incubation period is from 3 to 5 days, after which the larvae commence feeding on the leaf and prevent it from unrolling. The rolls become tighter as the growth of the leaf proceeds, and, as the larvae continue to feed, the leaf becomes hard and brittle and brown and may fall. There is little direct evidence of feeding other than the presence of small red blisters on the exterior of the rolled leaf.

The number of larvae per rolled leaf varies considerably. During February, twenty leaves collected at random, and averaging 4.5 cm. in length, were dissected. The number of larvae per single leaf varied from 147 to 491, and averaged 265.5. In April, a further twenty leaves averaging 3.8 cm. in length, were dissected. In this instance, the number of larvae per single leaf varied from 27 to 209, and averaged 92.8.

The feeding period of the larvae prior to pupating is about 3 weeks. On reaching maturity, the majority of larvae leave the trees by the ends of the rolled leaves, falling to the ground to pupate either among the fallen leaves or just below the surface of the ground. A small number of the mature larvae crawl down the trunks, and under these circumstances some of them pupate under the rough bark or any other shelter on the tree. Occasionally they pupate within the rolled leaf.

Rainfall softening the tightly rolled leaves influences movement of mature larvae, the largest numbers appearing to move from the leaves when the latter are thoroughly soaked by rain. Mature larvae, collected in the field and held in the laboratory, required a minimum of 14 days for pupal development.

Maximum numbers of adult midges were observed under warm calm conditions, but even under cool, overcast, windy conditions small numbers were still on the wing. On warm, sunny mornings the adults could be observed swarming approximately two feet above the ground and immediately below the trees, then, as if by a pre-arranged signal, the swarm would dissipate and the females commence oviposition. It is presumed that mating occurs during this swarming period. When confined to breeding cages, adults lived only a few days. The number of generations per season is still to be determined.

Prosactogaster demades Walker

This hymenopterous egg parasite, introduced to New Zealand in 1925 to control the pear leaf midge, *Dasyneura pyri* Bouché (Miller 1926, Dumbleton 1935), was first observed attacking *D. mali* during the

earlier part of the 1954-55 season. To ascertain the degree of parasitism, dissections of field-collected larvae were made at varying times throughout the summer and autumn. In many cases multiple parasitism occurred and in some instances as many as seven embryos were found in one host larva. In mid-January 1955, 50 larvae were dissected. Of these, 33 were parasitized, giving a level of parasitism of 66% with 10% multiple parasitism. In early February, 190 larvae from a single leaf showed 37% parasitism, with 9% multiple parasitism, and from another single leaf containing 404 larvae, 27% parasitism included five cases of multiple parasitism. At the end of March, a further 300 larvae, collected at random from the field, gave a figure of 66% parasitism, with 29% multiple parasitism.

The life cycle of *P. demades* is well synchronized with that of its host, the parasites emerging 2 to 3 days later than the adult midges. The parasite egg, which is colourless and extremely small, is normally located in the abdominal region of its host. When multiple parasitism occurs, some of the eggs are found in the thoracic region as well. The embryonic development of the parasite is prolonged, and free larvae were not found in the host until the latter had commenced spinning its cocoon. The parasite larvae become fully fed within a few days, during which time the host is destroyed.

DISCUSSION

As far as can be ascertained, there are no records of *P. demades* attacking *D. mali* either in England or on the Continent, where both the midge and the parasite are known to be present. Therefore, before considering the introduction of other parasites, the effectiveness of *P. demades* in controlling *D. mali* should be assessed locally.

There have been no serious outbreaks of *D. mali* in Europe, but in both the Argentine (Lopez Cristobal 1953) and the United States the midge is considered to be a pest of some importance. Whitcombe (1934) reports that in Massachusetts the number of larvae per single leaf varied from 9 to 30, averaging 17 with one heavily infested leaf containing 131 larvae. In New Zealand, Morrison (1953) stated that 28 larvae had been found in a single leaf. The levels of infestation reported by the above workers were considerably below that found at Palmerston North during the past season. As the degree of infestation provides an index to damage, a continuance of present population trends means that *D. mali* may become a pest of considerable importance not only to growers of apple stock but to the apple industry of New Zealand as a whole.

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NEW PUBLICATIONS

Two new Bulletins published by the Department of Scientific and Industrial Research.

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114. Trace element deficiencies of fruit crops in New Zealand by E. G. Bollard. Price 6/6.

In this bulletin the published information on trace-element deficiencies of fruit trees in New Zealand has been assembled together with some hitherto unpublished results of work carried out at the Fruit Research Station.

A general account is given of the occurrence of trace-element deficiencies, followed by a description of their detection and methods of treatment. In three separate sections on boron, manganese and zinc, the specific disorders caused by deficiency of these elements are discussed and illustrated in more detail.

115. Diseases and pests of peas and beans in New Zealand and their control. R. M. Brien, E. E. Chamberlain, W. Cottier, I. A. M. Cruickshank, D. W. Dye, H. Jacks, and W. D. Reid. Price 9/6.

The main leguminous crops grown in New Zealand for food consumption are peas, dwarf and runner beans, and broad beans. In this bulletin, diseases and insect pests commonly occurring on these host plants in the Dominion are described, and measures for their control recommended. The bulletin comprises sections dealing with fungous, bacterial, virus, and physiological diseases; insect pests; and the application of plant therapeutants.

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